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Cerebral processing and cortical plasticity during tonic and phasic painful stimulation

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Cerebral processing and cortical plasticity during tonic and phasic painful stimulation.

Ph.D. thesis

by

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Preface

This Ph.D. thesis is based on the four papers presented below in numerical order.

All these studies have been carried out in the time period of 2005-2008. Studies

1, 2, and 3 were carried out at the Cortical Plasticity and Human Brain Mapping Laboratory, Center for Sensory-Motor Interaction, Aalborg University, Denmark.

Study 4 was carried out at Dansk Hovedpine Center, Glostrup County Hospital, Copenhagen, Denmark. Studies 3 and 4 were carried out in collaboration with Dansk Hovedpine Center, Glostrup County Hospital, Copenhagen, Denmark.

Paper 1

Line Lindhardt Egsgaard, Li Wang, Lars Arendt-Nielsen. Volunteers with high versus low alpha EEG have different pain-EEG relationship: A human experimental pain study.

Exp Brain Res. 193 (2008): 361-369.

DOI: 10.1007/s00221-008-1632-1

Paper 2

Line Lindhardt Egsgaard, Li Wang, Lars Arendt-Nielsen. Gender Differences in Cuff-Pressure Pain: Psychophysics and High-Density EEG Power Mapping.

Submitted

Paper 3

Line Lindhardt Egsgaard, Line Buchgreitz, Li Wang, Lars Bendtsen, Rigmor Jensen, Lars Arendt-Nielsen. Short-term cortical plasticity can be induced by muscle pain.

Submitted

Paper 4

Line Buchgreitz, Line Lindhardt Egsgaard, Rigmor Jensen, Lars Arendt-Nielsen, Lars Bendtsen. Abnormal pain processing in tension-type headache: A high density EEG brain mapping study.

Brain 131 (2008): 3232-3238.

DOI: 10.1093/brain/awn199

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Cerebral processing and cortical plasticity during tonic and phasic painful stimulation.

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Abstract

In this thesis, the effects of experimental human pain on cerebral activation were investigated by use of both spontaneous EEG activity and somatosensory evoked potentials (SEP). Two pain models was used, tonic cuff-pressure (studies 1 and 2) pain and tonic glutamate evoked muscle pain with simultaneous phasic electrical stimuli (studies 3 and 4), to investigate the effects on human pain processing (and chronic pain, study 4). Significant findings in EEG frequency power analysis provided evidence for different pain-EEG relationship between high alpha vs. low alpha groups (H_{α} vs. L_{α}) and males vs. females. Study 1 showed clear differences between the H_{α} and L_{α} groups in alpha1 and alpha2 EEG powers but no differences in psychophysical responses to pain. In study 2, the male group had higher power in delta activity during pain and the female group had higher power in alpha2 and beta1, but no differences in psychophysical responses to pain. SEP and source analysis showed significant

findings between homotopic vs. heterotopic tonic pain and chronic tension type headache (CTTH) vs. healthy controls. Study 3 showed that the N100 peak latency increased during heterotopic tonic pain and the P200 peak latency increased during homotopic tonic pain. Homotopic and heterotopic tonic pain modulated the y-coordinate of the P200 dipole differently and specific changes in dipole localizations were found for homotopic and heterotopic tonic pain. In study 4, a significant reduction in magnitude during and after induced tonic muscle pain was found in controls at the P200 dipole whereas there were no differences found for patients. No consistent difference was found in localization or peak latency of the dipoles. Taken together, we conclude that (a) EEG frequency power analysis can reflect differences in pain processing between two diverse groups, (b) heterotopic tonic muscle pain causes local changes in cortical processing and homotopic tonic muscle pain causes general and long-lasting changes in cortical processing, and (c) CTTH patients have impaired inhibition of nociceptive inputs.

Key words: Experimental human pain, EEG, tonic pain, somatosensory evoked potentials

Danish summary

Forord

I denne afhandling undersøges effekten af eksperimentel menneskelig smerte på cerebral aktivering ved brug af både spontan EEG aktivitet og somatosensoriske evokerede potentialer (SEP). To smertemodeller benyttes, tonisk manchetrykalgometri (studier 1 og 2) og tonisk glutamat evokeret muskel smerte med samtidig fasisk elektrisk stimulering (studier 3 og 4), til undersøgelse af effekten på menneskelig smerteprocessering. Signifikante fund i EEG frekvens analyse viste forskellige smerte-EEG forhold mellem høj alfa vs. lav alfa grupper (H_α vs. L_α) (studie 1) og mænd vs. kvinder (studie 2). Studie 1 viste klare forskelle mellem H_α og L_α grupper i alfa1 og alfa2 EEG styrke men ingen forskelle i psykofysiske responser til smerte. I studie 2, havde gruppen af mænd højere styrke i delta EEG aktivitet under smerte og den kvindelige gruppe havde højere styrke i alfa2 og beta1 EEG styrke, men ingen forskelle i psykofysiske responser til smerte. SEP og cerebral positions analyse viste signifikante forskelle mellem homotopisk vs. heterotopisk tonisk smerte (studie 3) og mellem kronisk spændingshovedpine (CTTH) vs. raske kontroller (studie 4). Studie 3 viste, at N100 latenstid forøges under heterotopisk tonisk smerte og P200 latenstiden forøges under homotopisk tonisk smerte. Homotopisk og heterotopisk tonisk smerte modulerede y-koordinaten af P200 dipolen forskelligt, og specifikke skift i dipollokaliseringer blev fundet for homotopisk og heterotopisk tonisk smerte. I studie 4 blev der fundet en signifikant reduktion i dipolstyrke ved P200 dipolen

under og efter induceret tonisk muskel smerte, hvorimod der ikke blev fundet nogle forskelle for patienter. Der var ingen konsistente fund i lokalisation eller latenstid for dipolerne hverken for patienter eller kontroller. Sammenfattet konkluderer vi, at (a) EEG frekvens styrke analyse kan reflektere forskelle i smerteprocessering mellem to uens grupper, (b) homotopisk, men ikke heterotopisk tonisk muskelsmerte fremkalder detekterbar kort-tids kortikal plasticitet efterfølgende repetitiv intramuskulær elektrisk stimulering, og (c) CTTH patienter har svækket hæmning af smertefulde inputs.

Nøgleord: Eksperimental menneskelig smerte, EEG, tonisk smerte, somatosensoriske evokerede potentialer

1. Introduction

1.1. *Pain*

1.1.1 Pain physiology (nociception)

Pain sensation (pricking, burning, aching, stinging, and soreness) is a protective somatic sensation which warns of potential injury. Pain has an urgent and primitive quality and is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP Definition of Pain). Pain is divided into pain perception (the experience of pain) and nociception (the neural mechanisms). Nociceptors (thermal, mechanical and polymodal) are activated by harmful stimuli to the skin, joints and muscles and are mediated by thinly myelinated A δ -fibers (first pain, thermal and mechanical nociceptors) and unmyelinated C-fibers (second pain, polymodal nociceptors) which terminate in the superficial layers of the dorsal horn (first order neurons). The dorsal horn neurons send their axons across the midline of the spinal cord and ascend contralaterally in the spinothalamic tract of the anterolateral column directly to the thalamus. In the thalamus third-order neurons send axons to the primary somatosensory cortex (SI) which interacts with the secondary somatosensory cortex (SII) which again projects to the insular cortex and other subcortical structures (Kandel et al., 2000) resulting in the feeling of pain. The somatosensory cortices are responsible for the perception of sensory features such as the location and duration of pain, whereas the limbic and paralimbic

structures (e.g. anterior cingulate cortex, insular cortex) are involved in the emotional and motivational aspects of pain (pain perception) (Kandel et al., 2000).

1.1.2 Pain perception.

Nociception does not necessarily lead to pain perception. Pain perception is the affective and emotional aspect of pain which is a product of the brain's abstraction and elaboration of sensory input (Kandel et al., 2000). Pain perception normally varies among individuals and depends of the mental state of the individual. Attention, anxiety, fear, and sociocultural factors can modulate the pain experience (Staehelin Jensen et al., 2003).

Increased attention towards pain (hypervigilance) causes an intensified pain sensation whereas distraction from pain decreases the pain sensation; distraction only possible during short-lasting pains whereas hypervigilance towards pain is usually developed in recurring and chronic pain states (Staehelin Jensen et al., 2003).

Anxiety is the feeling of uncontrollability and unpredictability and future-oriented mental state where one is prepared to attempt to cope with upcoming negative events (Barlow, 1991). Anxiety is associated with distortions in information processing and results in disruption of concentration and performance (Barlow, 1991). The level of anxiety during pain (as measured by pain anxiety symptoms scale) has shown to have a negative effect on the perceived pain (Kandel et al., 2000);

Fear is a primitive “fight-or-flight” response; fear of pain causes individuals to selectively attend towards pain related material (words, dot-probe test) and may be vulnerability factor which predisposes individuals to react more negatively towards pain (Keogh et al., 2001b).

Sociocultural factors such as gender, age, nationality, and past pain experiences affect the pain experience, according to the gate-control theory, because attitudes, expectations, meaning for experiences, and appropriate emotional expressiveness are learned through observation of other who are similar to in identity to oneself (Bates, 1987).

Pain is a complex perception which is influenced by many factors and the context in which the nociceptive input occurs and it involves a complex cortical network.

1.1.3. Pain-related brain structures

Pain experiences are divided into four components: sensory, motor, affective/emotional and autonomic.

Neurons in selective areas in the cortex respond to nociceptive inputs after relay in the thalamic sensory nuclei. These areas include primary somatosensory cortex, premotor area, secondary somatosensory cortex, insula and cingulate cortex (Niddam et al., 2005). The primary somatosensory cortex has a somatotopical representation of the body. The area dedicated to processing information from a particular part of the body becomes active when noxious inputs are received from that specific part. The primary somatosensory cortex sends inputs to the prefrontal cortex and the premotor cortex to prepare and

select the appropriate movement which is then projected to the primary motor cortex. The prefrontal, the premotor and the primary motor cortices constitute the **motor** component of pain perception. The neurons of the primary somatosensory cortex innervate the secondary somatosensory cortex which contain neurons that code spatial, temporal and intensive aspects of noxious (and innoxious) stimuli. The primary and secondary cortices constitute the **sensory** component of pain perception. The secondary somatosensory cortex projects to the insular cortex which process information of the internal state of the body contributing to the **autonomic** component of the overall pain response. The cingulate cortex together with the frontal lobes, amygdala, hypothalamus and the brainstem is responsible for the conscious feeling/emotion constituting the **affective/emotional** component of the pain experience.

1.1.4. Pain pathophysiology

Pain can be acute or chronic. Acute pain is short lasting and usually disappears when treated while chronic pain is long lasting and does not respond well to treatment. It is believed that cerebral plasticity, a so-called central sensitization is the cause of many chronic pain syndromes. Central sensitization can be induced by frequent nociceptive inputs and it is defined as an increase in excitability of spinal neurons (Woolf, 1983). It manifests as an abnormal or heightened sensitivity and the generation of pain by low activation of A β mechanoreceptors (Kandel et al., 2000; Herrero et al., 2000). Three terms are commonly used for pain pathophysiology caused by central sensitization: allodynia, hyperalgesia and

neuropathic pain. Allodynia creates a painful sensation to non-painful stimuli but does not lead to pain in the absence of stimulus. Hyperalgesia is a condition where spontaneous pain occurs and noxious stimuli create an excessive response. Neuropathic pain is constant or persistent and a result of direct injury to the nerves and is often characterized by a burning or electric sensation. Lowered pain sensation (i.e. higher pain thresholds) to painful stimuli is termed hypoalgesia and analgesia. Hypoalgesia is a decreased sensitivity to painful stimuli and analgesia is the loss pain sensation both of which are caused by an interruption in the nervous system pathway between periphery and brain.

1.2. Human experimental pain research

Experimental pain is evoked in validated models mimicking aspects of acute pain (phasic pain) or chronic pain (tonic pain). These pain models are safe and include thermal, mechanical, chemical, electrical stimulation paradigms which produce reliable and meaningful data.

1.2.1. Phasic and tonic pain

Experimental pain is classified into phasic or tonic pain according to the duration of pain. Short-lasting phasic pain reflects the immediate impact of the onset of injury. Phasic pain is intrinsic pain-specific discomfort which triggers fear and anxiety (Wall and Melzack, 1999). In experimental settings, phasic pain stimulation can be applied to skin, muscle and viscera by electrical, tactile, and thermal stimulation.

Long-lasting tonic pain persists or increases for a variable time period until stimulation stops or the effects of the stimulation disappears. In experimental settings, tonic pain can be applied to skin, muscle and viscera by electrical, tactile, thermal and chemical stimulation.

1.3 Electroencephalogram (EEG) and pain

Since Hans Berger (1933) first recorded EEG from man; the technology and analysis of EEG has become very advanced and has been used in basic research as well as clinical settings. EEG is complex signals which change over time and have different properties depending on the place over the head where they are recorded. EEG allows non-invasive access to brain processes at an integrative level of the central nervous system with high degree of spatio-temporal resolution by use of high-density recording and interpolation. EEG dynamically reflects the cerebral function with co-activation of the different regions of the brain and it is now regarded that only high-density EEG can provide sufficient temporal as well as spatial resolution of brain activation. These signals can be analyzed with various methods which can be divided into two categories: nonparametric and parametric methods. Two methods have been used in this thesis; (1) (nonparametric) frequency analysis (power spectra) and (2) (parametric) source analysis (inverse problem).

1.3.1. EEG frequency analysis and pain

Frequency analysis is a classical way of describing the EEG signals. Fourier analysis and the common EEG frequency bands are used to obtain information from the frequency components of the EEG signals. The EEG frequency bands are typically comprised of 7 bands; delta (0.5-3.5Hz), theta (4.0-7.0 Hz), alpha1 (7.5-9.5 Hz), alpha2 (10-12 Hz), beta1 (13-23 Hz), beta2 (24-34 Hz), and gamma (35-45 Hz). 3D topographic maps (plots) on a head model display the power distribution of the brain activity (as measured from the surface of the scalp). In response to tonic pain relatively consistent changes in EEG frequency bands have been found; (a) increase in low frequency delta power; (b) rare change in theta power; (c) decrease in alpha power; and (d) increase in beta power (for reviews see Chen, 2001; Bromm and Lorenz, 1998).

1.3.2. Somatosensory Evoked Potentials (SEPs) and pain

The ultimate goal of EEG potentials recorded at the scalp is to find the intracranial sources. The intracranial sources can be determined by solving the “inverse problem” from the distribution of evoked potentials at the scalp. Evoked potentials are the electrical signals generated by the nervous system in response to sensory stimuli. These time-locked electrical signals are analyzed according to the amplitude and peak latency from which the intracranial sources can be computed by using the model of the volume conductor (brain, cerebral spinal fluid, skull and scalp).

The early painful SEP components (20ms -50 ms) are the somatotopic projection to the primary sensory cortex (Allison et al., 1989) and are elicited by fast

myelinated A δ fibers. The middle components (50ms – 200ms) are diffuse distributed but they have been suggested to be compatible with A δ myelinated fibers (Babiloni et al., 2001). The late components (200ms - 300ms) could partly be related to A δ fiber and partly non-myelinated C-fiber activation (Chen, 2001) and are typically located around the cingulate cortex (Bromm and Lorenz, 1998).

1.4. Aim of the Ph.D.

Neuro-imaging has been used extensively to investigate the cerebral activation of human pain (for review see e.g. Chen, 2001; Apkarian et al., 2005). Two EEG analysis techniques were used to assess the cerebral processing of pain. Two basic studies (study 1 and study 3) and two applied studies (study 2 and study 4) were conducted employing two different experimental pain models (tonic and phasic pain) and psychophysical evaluation. Tonic pain was used as a pain model in all four studies; in studies 1 and 2 a tonic cuff-pressure pain model was used and in studies 3 and 4 intramuscular injection of glutamate was used. In studies 3 and 4 electrical phasic pain was applied in conjunction with the tonic pain model. The logical outline of the project is illustrated in Figure 1. The aims of the four studies are described below:

Study 1: The aim of this study was to examine the effect of tonic pain stimulation on occipital alpha EEG activity during different levels of pain. It was investigated if high versus low alpha groups have different pain reactions and pain-EEG relationships.

Study 2: The aims were to study the gender differences in (1) cuff pressure pain and distress ratings, and (2) the evoked ongoing EEG activity and its topographical distribution.

Study 3: This study aimed to identify (1) short-term cortical plasticity before; during and after glutamate evoked tonic pain or sham stimulation and (2) short-term cortical plasticity evoked by different sites of the glutamate induced tonic muscle pain.

Study 4: The aim of this study was to identify differences in dipole components (peak latency, magnitude, localization) CTTH patients and controls before, during and after glutamate evoked tonic pain in response to single and repeated phasic electrical stimuli. Further, differences in quantitative sensory parameters between patients and controls were assessed.

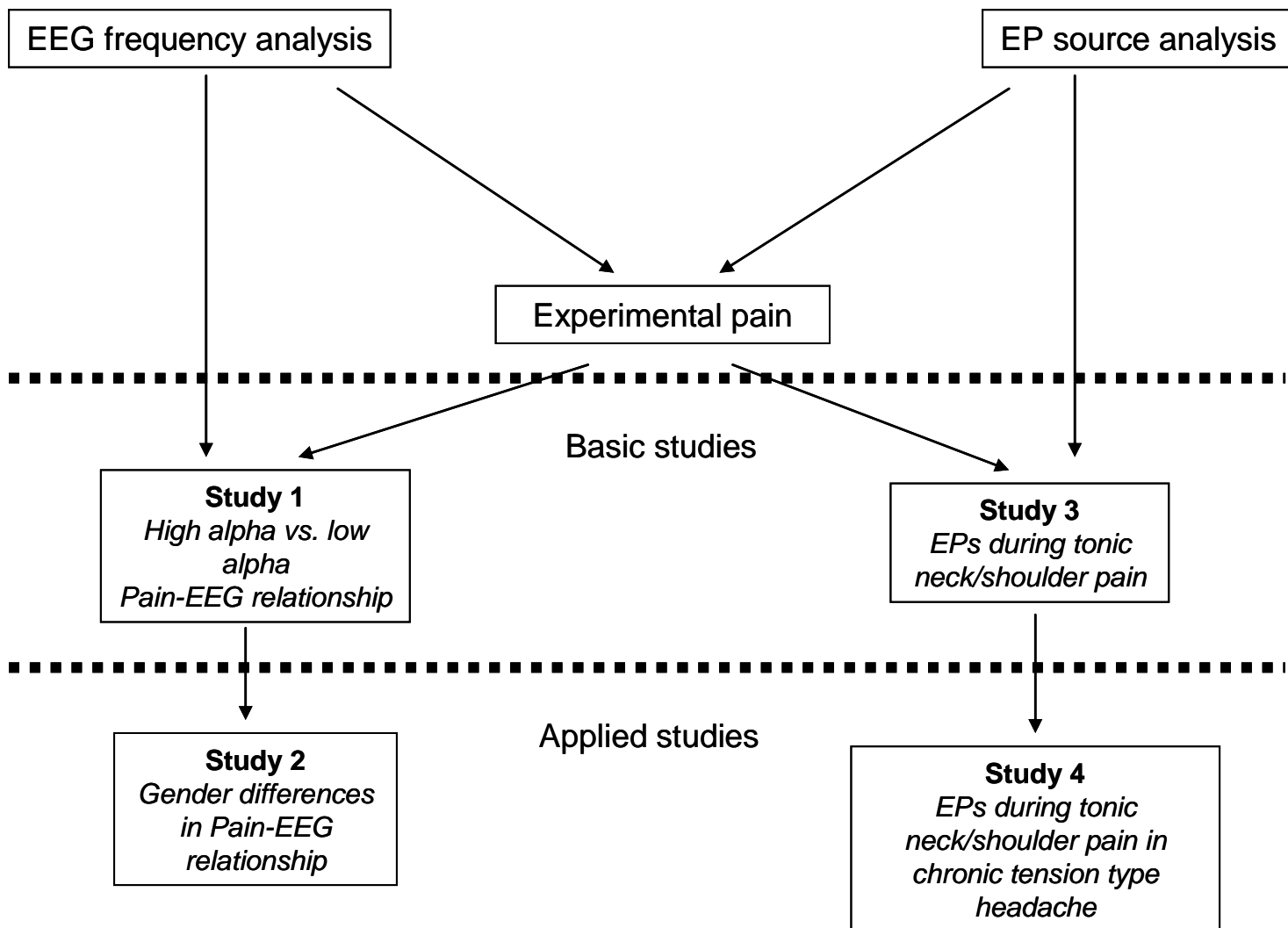


Figure 1: Outline of the Ph.D. project.

2. Experimental pain models

Phasic and tonic pain models are applied in experimental settings with different purposes. Clinical pain is persistent and recurring which is modeled with a tonic pain paradigm whereas phasic pain is typically used to evoke spinal or cortical responses. In this thesis, two pain stimulation paradigms were used; tonic cuff-pressure stimulation (studies 1 and 2) and glutamate evoked tonic muscle pain with simultaneous phasic intramuscular electrical stimuli (studies 3 and 4).

2.1. Tonic cuff-pressure stimulation

Mechanical pressure is an established method for estimation in normal and sensitized muscles. Mechanical pressure (pressure pain thresholds) is used to study and as diagnostic tool in musculoskeletal pain syndromes such as fibromyalgia, myofascial pain, temporomandibular disorder and tension type headache (for review see Treede et al., 2002). These musculoskeletal pain syndromes exhibit lower pressure pain thresholds in so-called tender and/or trigger points.

Pneumatic cuffs are used in clinical settings for arterial pressure measurement and tourniquet application in surgery. Tourniquets (cuffs) are used in pain research to study and evaluate ischemia (Torebjork and Hallin, 1973). Cuff-pressure directly activates mechanoreceptors of all tissues under the cuff; however, the pain is deeply located. When tonic cuff-pressure is applied to humans the pain increases with time (ischemic pain) (Wall and Melzack, 1999). Thus it appears that C-fiber afferents are involved in tonic pressure pain.

Previous EEG studies have consistently demonstrated systematic changes in specific frequency bands during experimental tonic pain; a decrease in alpha power (7.5 Hz – 12 Hz) and increase in beta power (13 Hz – 34 Hz) have been suggested to be pain specific (Chang et al., 2002a; Chang et al., 2002b; Chang et al., 2003; Chang et al., 2004; Chang et al., 2001).

2.2. Glutamate evoked tonic muscle pain

Artificial elevation of glutamate (NMDA receptor) concentration by injection of the excitatory amino acid glutamate induces mechanical allodynia (sensitization) and the duration of the muscle sensitization is considerably longer than the duration of the acute pain from the injection itself (Svensson et al., 2003). Injection of glutamate usually generates short-term muscle hyperalgesia to pressure stimulation (Svensson et al., 2003; Arendt-Nielsen et al., 2008; Cairns et al., 2002; Cairns et al., 2003). Injection of glutamate in the rat masseter muscle activates peripheral NMDA (N-methyl-D-aspartate) and/or non-NMDA receptors (Cairns et al., 2003). NMDA receptors participate in the windup of dorsal horn neurons (Dougherty et al., 1992). Windup is believed to be one of the triggers of central sensitization (Woolf and Thompson, 1991; Woolf, 1996) and NMDA receptors are reported to play a role in the maintenance of central sensitization (Dickenson et al., 1997; Carlton, 2001).

2.3. Phasic intramuscular electrical stimulation

Intramuscular electrical stimulation (IMES) evokes sensory and motor fibers within the muscle and is used for functional purposes such as functional electrical

therapy (FES) and neuroprostheses. A limited number of studies have investigated IMES somatosensory evoked potentials. The disadvantage of IMES is that muscle twitches are evoked and that the stimulus activates both nociceptive and non-nociceptive afferents (Laursen et al., 1999). However, non-specific intra-muscular electrical stimulation (IMES) has been used in experimental studies to investigate cortical plasticity, by use of somatosensory evoked potentials (SEPs), related to muscle pain (Niddam et al., 2005; Niddam et al., 2001; Niddam et al., 2007; Niddam et al., 2008; Svensson et al., 1997). SEPs from intra-muscular electrical stimulation do not elicit detectable early SEP components (< 80 ms) (Niddam et al., 2005) but generates larger mid-latency components (Shimojo et al., 2000). SEPs from repeated painful muscle stimulation, as compared to single stimulation, decrease in amplitude at 100 ms (N100) and 250 ms (P250) and the P450 peak disappears (Chen et al., 2000). Dipole source reconstruction techniques, based on high resolution SEP recordings, have been used to identify cortical areas involved in pain processing of electrically evoked muscle pain (Niddam et al., 2005). The areas activated include primary sensorimotor area, premotor area, secondary somatosensory area, insula and cingulate cortex (Niddam et al., 2005). Functional imaging studies (PET, fMRI) (Niddam et al., 2007; Niddam et al., 2008; Svensson et al., 1997; Niddam et al., 2002) of experimentally evoked muscle pain have found additional activity in the thalamus, parietal cortex, lenticular nucleus, superior temporal gyrus, supplementary motor gyrus, precuneus, claustrum, caudate and putamen.

3. Methods

3.1. Pain ratings

The Verbal Rating Scale (VRS) was used in all 4 studies and was defined as: 0= no change (in pain perception), 1= barely intense, no pain, 2= intense, no pain, 3= fairly intense, but no pain, 4= slight pain (pain-threshold), 5= mild pain, 6= moderate pain, 7= moderate-strong pain, 8= strong pain, 9= severe pain, 10= unbearable pain.

3.2. EEG Data Acquisition

The EEG was recorded from 128 surface electrodes including two EOG (Electro OculoGram – voltage difference between the cornea and retina) channels and two mastoid reference channels using a standard EEG-cap (Waveguard cap system, Cephalon A/S) employing the 10-5 montage system (Oostenveld and Praamstra, 2001). Bipolar EOG was recorded, horizontal EOG was measured with tin electrodes attached to the outer canthus of each eye, and vertical EOG was recorded from supra-orbital electrodes placed in line with the pupil of the right and left eye, so that the portion of EOG contamination of each scalp trace could be removed offline. Impedance was kept below 5 K Ω . EEG signals were sampled at 512 Hz for studies 1 and 2 and 2048Hz for studies 3 and 4. Sixteen bit resolution in EEG quantification was used. The EEG was recorded by use of the EEProbe Software (ANT-Software A/S, Netherlands).

3.2. Studies 1 and 2: tonic pressure stimulation

3.2.1. Experimental Procedures

A single tourniquet cuff and manometer (up to 600 mm/Hg) with hand inflator (Braun Scandinavia A/S Copenhagen, Denmark) was used to induce tonic cuff-pressure pain (Polianskis et al., 2002b; Polianskis et al., 2002c; Polianskis et al., 2002a; Polianskis et al., 2001) in the upper right arm. Before the experiment started, all three pressure levels corresponding to VRS2 (intense, but no pain), VRS4 (pain threshold) and VRS6 (moderate pain) were identified by averaging 5 ascending trials separated by 1 min. The pressure level detection was implemented by pumping the hand inflator every 2 seconds until the subject indicated that the pain level was reached.

The experiment consisted of a resting baseline EEG (2 min with eyes closed and 2 min with eyes open) and three experimental conditions with pain levels corresponding to the Verbal Rating Scale (VRS) 2, 4, and 6 pain levels each maintained for 3 minutes. The experimental conditions were performed in the following order: baseline (2 min eyes closed), baseline (2 min eyes open), tonic cuff-pressure pain VRS2, VRS4 and VRS6 (performed in this order) with a 5 minute rest period between the experimental conditions. The subjects were instructed to stop anytime during the experiment if it was too unpleasant. During each experimental condition, EEG (128 channels) was recorded while the subjects held their eyes closed. The subjects rated their pain verbally every 15 seconds on the VRS scale over the 3 min stimulation period to measure subjective pain intensity changes over time.

3.2.3. Analysis of EEG Data

The EEG was band pass (0.5 Hz – 100 Hz) and notch (50 Hz) filtered and divided into 2 second epochs. The epochs were subjected to automatic artifact rejection (above +80 and below -80 μ V) followed by visual artifact rejection on the remaining epochs. The valid epochs were subjected to Fast Fourier Transform in order to produce the power density. Bad electrodes were detected and interpolated in the frequency domain with the four neighboring electrodes located on the anteroposterior axis and the mediolateral axis from the bad electrode (bad electrodes located on the edge of the electro cap were interpolated with 3 neighboring electrodes). The EEG powers were group averaged in baseline and each experimental condition in order to identify the activation area in each broad band.

3.2.4. Focal Areas

The focal areas consisting of the focal maximum and the 4 neighboring electrodes (total area at 9.9 cm² given the inter-electrode distance at 3.0 x 3.3 cm of the 10-5 system) were extracted from the groups (study 1: H α and L α ; study 2: male and female). Bilateral electrodes were chosen for all frequency bands; except for the theta band where focal maximum was located central. The following focal maxima were chosen for analysis in study 1 (expressed by band(electrode)): alpha1(PO3), alpha1(PO4), alpha1(PO7), alpha1(PO8), alpha2(PO3), alpha2(PO4). In study 2 all EEG frequency bands were analyzed hence additional focal maxima were chosen: delta(AF7), delta(AF8), delta(Fp1),

delta(Fp2), theta(FCz), beta1(PO3), beta1(PO4), beta2(T7), beta2(T8),
gamma(T7), gamma(T8).

3.2.5. Correlation between EEG power and subjective ratings

The average subjective rating for each subject was calculated over the 3 min period for VRS2, VRS4 and VRS6 to have one pain rating describing the experimental condition (12 pain ratings were recorded for each experimental condition and pain increased over time). The average subjective pain rating for experimental conditions VRS2, VRS4 and VRS6 for each subject was paired with the corresponding EEG power in the each focal area.

3.2.6. Statistical Analysis

Cuff-pressure levels and pain ratings were analyzed with a t-test to determine differences between the high alpha (H_α) vs. the low alpha (L_α) groups (study 1) and the male vs. female groups (study 2). Analyses to identify EEG differences and responses to tonic pain between the H_α and L_α were conducted with a Two Way RM ANOVA (factor A: intensity; factor B: group) on the EEG power change relative to baseline (subtracting the EEG recorded during baseline from that recorded during the VRS2, VRS4, and VRS6 tonic cuff-pressure conditions). Graphical representations of EEG changes are expressed in relative power (%) in respect to baseline.

Analyses to identify EEG differences between the male vs. female were conducted with a Two Way RM ANOVA (factor A: intensity; factor B: gender). All statistical analysis on EEG was conducted with log-transformed values to

enhance the normality distribution in the EEG. The results were expressed in mean values \pm SE. The SigmaStat 2.03 program was employed and $p < 0.05$ was considered a significance. A post-hoc Tukey HSD test was employed to verify the significance and correction for multiple comparisons.

Correlations between EEG power and the corresponding average subjective pain ratings were calculated with linear regression for the H_{α} , the L_{α} and with Pearson's correlation for the male and female groups separately in each focal area.

3.3. Studies 3 and 4: intramuscular electrical stimulation and tonic muscle pain

3.3.1. Experimental Procedures

The subjects were asked for demographic data (weight, height, age, hand orientation) and were seated in a hospital bed. Before the experiment, the subjects were familiarized with the electrical stimulation and injection procedures. The reference point on trapezius was marked 2 cm lateral to the halfway point between the spinous process of the seventh cervical vertebra (C7) and the lateral edge of the acromion. The needle electrodes (Medtronic, disposable sensory needle electrode, 20mm x 0.35mm (28G), recording area 2.0 mm²) were placed with a 10 mm distance in a 5 mm depth in the muscle. The electrodes were placed 5 mm anterior and 5 mm posterior to the reference point. Electrical pain thresholds for the single stimulation (PT_{single} , duration of 1ms) and repeated stimulation (PT_{repeated} , 5 pulses, 1 ms duration, repeated with 2Hz)

(Chen et al., 2000) were determined by method of limits. PT_{single} and PT_{repeated} were measured 3 times before each session with 1 min interval, starting from 0 mA and increasing slowly with 0.1 mA steps. The electrical stimuli intensities were constant in individual subjects in all experimental conditions (in study 3 stimulation intensities were constant in one session). Measurement of PT_{single} and PT_{repeated} was repeated approximately 20 minutes post-injection (post- PT_{single} and post- PT_{repeated}).

Constant current electrical stimulation (NoxiTest Biomedical A/S, Aalborg, Denmark) was controlled and programmed with LabVIEW (National Instruments). Electrical stimuli (60 single and 60 repeated stimuli) were given in randomized order with inter stimulus interval between 4 and 6 sec. Single stimuli were given at the PT_{single} intensity and train stimuli were given at the PT_{repeated} intensity.

3.3.2. Injection procedures

The injection (0.2 ml of glutamate (L-monosodiumglutamate 1M, 1mmol – 187 mg, 2 ml) or isotonic saline (isotonic saline 0.9 %, 2 ml - only for study 3)) was given with 1 ml syringe and a 27 G X 3/4 inch cannula. The injection site of trapezius was in the center between the two intramuscular stimulation electrodes and in the thenar (only for study 3) the injection site was in the muscle belly. The subjects rated the perceived tonic muscle pain intensity on the VRS scale every 30 seconds until the pain disappeared. When the pain rating fell below 4 on the VRS scale, another glutamate injection was given. For study 3 in the control

(sham pain) session, two isotonic saline injections were given, one 1 min prior to the SEP recording and one 5 min after SEP recording started.

The experiment (each session in study 3) consisted of 3 experimental conditions, (1) baseline recordings (pre injection SEP recording), (2) tonic pain SEPs with simultaneous glutamate injection (or isotonic saline (study 3)), and (3) post-baseline recordings (post injection SEP recording). The stimulation period was approximately 10 min for each experimental condition. Each experimental condition was followed by a 5-10 min break or until the pain disappeared.

3.3.3. Analysis of EEG Data

Epoching, artifact rejection, and averaging were performed by use of custom made Matlab/LabVIEW based software. Single sweeps were cut into epochs with a length of 700 ms, 100 ms before and 600 ms after the stimulus onset. The repeated sweeps were cut into 5 separate (repeat(1-5)) epochs of 600 ms, one epoch for each of the 5 stimuli, 100 ms before and 500 ms after the stimulus onset and they were analyzed separately. The single pulse SEP, 1st (repeat(1)) and the 5th (repeat(5)) stimuli of the repeated SEP were analyzed.

The epochs for single, repeat(1) and repeat(5) were forward and reverse filtered with 4th order Butterworth band pass filter (0.5-100Hz) in Matlab 7.0. All epochs were transformed to a common average reference offline. Artifact rejection was done by visual inspection on each epoch and the valid epochs in each experimental condition for each subject were averaged. This average represented the SEP. Each SEP was further processed with the Matching Pursuit

algorithm (Mallat and Zhang, 1993; Gratkowski et al., 2006; Gratkowski et al., 2008) which decomposes the signal into frequency components. These components can be enabled or disabled and thus the 50 Hz component and any other outer and/or inner disturbances can be eliminated and thereafter the SEP can be recreated. Bad electrodes were detected and interpolated with the four neighboring electrodes (bad electrodes located on the edge of electrocap was interpolated with 3 electrodes) located on the anteroposterior axis and the mediolateral axis from the bad electrode.

Peak latencies around 100 ms (N100), 200 ms (P200), and 300 ms (P300) were extracted for each SEP from the compressed waveform (butterfly plot). The corresponding current dipole components were computed with the moving dipole model for the 3 peak latencies (N100, P200, P300). The dipole coordinates x, y, z are expressed in the Subjects Coordinate System as provided by the manufacturer (ANT-Software A/S, Netherlands); where the positive x-axis is directed toward the nasion, the positive y-axis is directed toward the left pre-auricular point, and the positive z-axis is directed toward the vertical central parietal. The calculated dipole was superimposed on MRI slices of the MNI standard brain. Topographic maps and source analysis was performed with commercial available software ASA 3.0 (Advanced Source Analysis, ANT-Software A/S, Netherlands) and dipole MRI maps created with BrainVoyager Brain Tutor 2.0 (© 2003-2007 Rainer Goebel, <http://www.brainvoyager.com/BrainTutor.html>).

3.3.4. Statistical Analysis

A paired t-test was employed at 0 sec, 300 sec, and 600 sec after injection to test for pain adaptation or sensitization during tonic pain/sham pain (sham pain was only studied in study 3) (VRS score) and VRS score differences between the two groups in the sham pain (study 3) and glutamate conditions (study 3 and 4). Pre- and post injection pain thresholds were compared with a paired t-test. Pain thresholds (PT_{single} and PT_{repeated}) differences between the two groups were tested with a Two Way RM ANOVA (factor A: injection substance, factor B (study 3): muscle, factor B (study 4): patient/control). Differences in SEP and dipole components (peak latency, x, y, x, magnitude) were tested with a Two Way RM ANOVA (factor A: experimental condition, factor B (study 3): muscle, factor B (study 4): patient/control). Accordingly, 'condition' x 'group' interaction and 'group' effect (difference between the two groups when all conditions were analyzed together) were analyzed. The SigmaStat 2.03 program was employed and $p < 0.05$ was considered a significance. A post-hoc Tukey HSD test was employed to verify the significance and correction for multiple comparisons. The results were expressed in mean values \pm SE.

4. Results

4.1. High vs. low alpha EEG in response to tonic pressure pain (study 1)

4.1.1. Group separation

Study 1 divided 40 subjects into high (H_{α}) and low (L_{α}) alpha groups based on the median split of total occipital alpha EEG activity at baseline (Figure 2). subjects

with total occipital alpha EEG activity above $600 \mu V^2$ was in the H_α and subjects with total occipital alpha EEG activity below $600 \mu V^2$ was in the L_α . The H_α consists of 14 females and 6 males; the L_α consists of 6 females and 13 males.

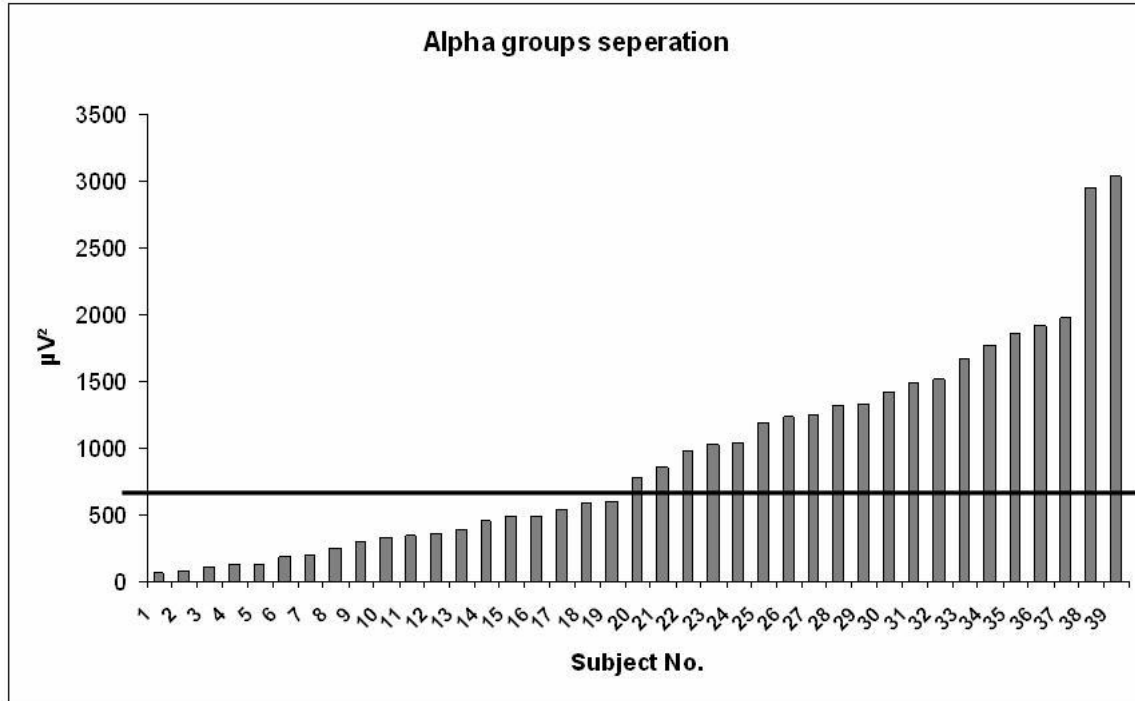


Figure 2: The alpha (alpha1 + alpha2 EEG power at baseline) power in all subjects. The line illustrates the separation of subjects into high alpha (H_α , above the $600 \mu V^2$ line) and low alpha (L_α , below the $600 \mu V^2$ line) groups.

4.1.2. H_α and L_α differences in EEG power

The patterns of EEG topography (absolute power in μV^2) in the alpha1 and alpha2 bands for H_α and L_α groups are illustrated in Figure 3. Alpha1 activity is 4 folds ($40 \mu V^2$ versus $10 \mu V^2$) higher in the H_α (left side Figure 3). Maximal alpha2 activity is 5 folds larger ($100 \mu V^2$ versus $20 \mu V^2$) in the H_α . Differences in alpha1 EEG changes relative to baseline between the H_α and the L_α groups have been detected in alpha1(PO3) ($F=10.933$, $P=0.002$, post hoc=0.002), alpha1(PO4)

($F=11.978$, $P=0.001$, post hoc= 0.001), alpha1(PO7) ($F=9.734$, $P=0.003$, post hoc= 0.004), and alpha1(PO8) ($F=10.866$, $P=0.002$, post hoc= 0.002). Further, differences between the H_α and the L_α groups were identified in alpha1 EEG power changes relative to baseline in all experimental conditions; VRS2 (alpha1(PO3): post hoc= 0.032 ; alpha1(PO4): post hoc= 0.034 ; alpha1(PO7): post hoc= 0.041 ; alpha1(PO8): post hoc= 0.038) VRS4 (alpha1(PO3): post hoc= 0.007 ; alpha1(PO4): post hoc= 0.003 ; alpha1(PO7): post hoc= 0.009 ; alpha1(PO8): post hoc= 0.004) and VRS6 (alpha1(PO3): post hoc= 0.001 ; alpha1(PO4): post hoc ≤ 0.001 ; alpha1(PO7): post hoc= 0.002 ; alpha1(PO8): post hoc= 0.002).

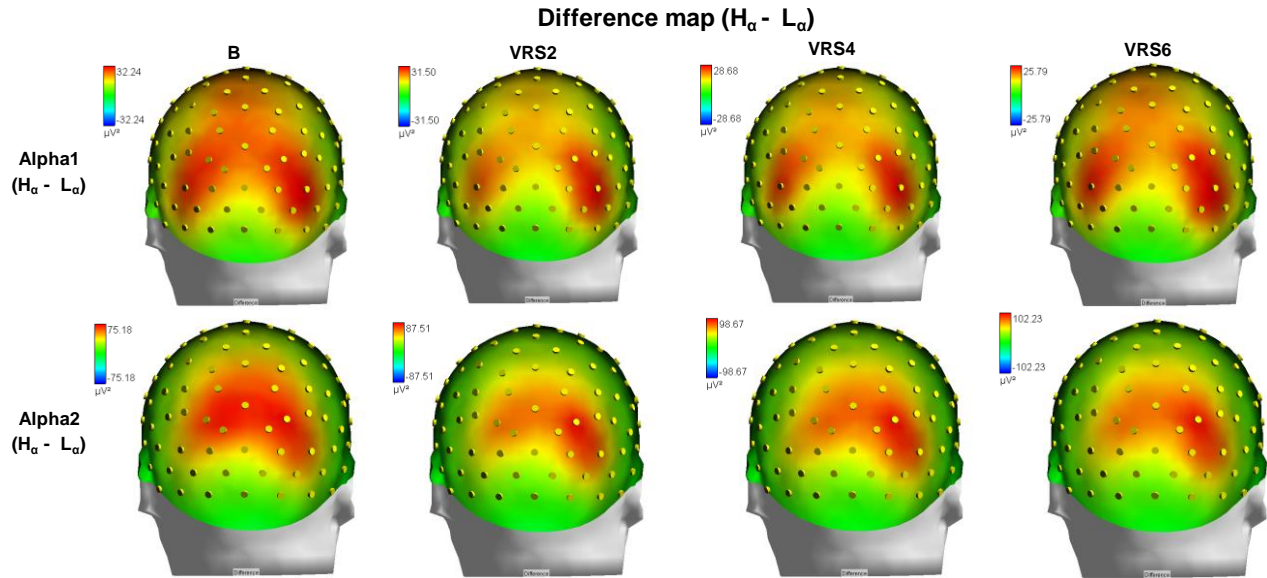


Figure 3: Difference map showing the clear differences between the H_α and the L_α groups in the alpha1 and alpha2 EEG bands. The difference map was created by subtracting the L_α from the H_α group (absolute power maps for L_α and the H_α are illustrated in study 1). The differences between H_α and L_α subjects are illustrated in red (positive difference= H_α have higher power) and blue colors (negative difference= L_α have higher power). B = baseline, VRS2 = non-painful pressure level, VRS4 = slightly painful pressure level, pain threshold, and VRS6 = moderately painful pressure level.

4.1.3. Changes within H_α and L_α

The L_α group desynchronizes from baseline to VRS2 and desynchronization

decreases as pain increases, whereas the desynchronization for the H_α group

increases as pain increases (Figure 4). Additionally, the H_a group shows an increase in $\alpha_2(PO3)$ EEG power changes relative to baseline from experimental conditions VRS2 to VRS6 ($\alpha_2(PO3)$: $F=3.634$, $P=0.031$, post hoc=0.009) (Figure 5).

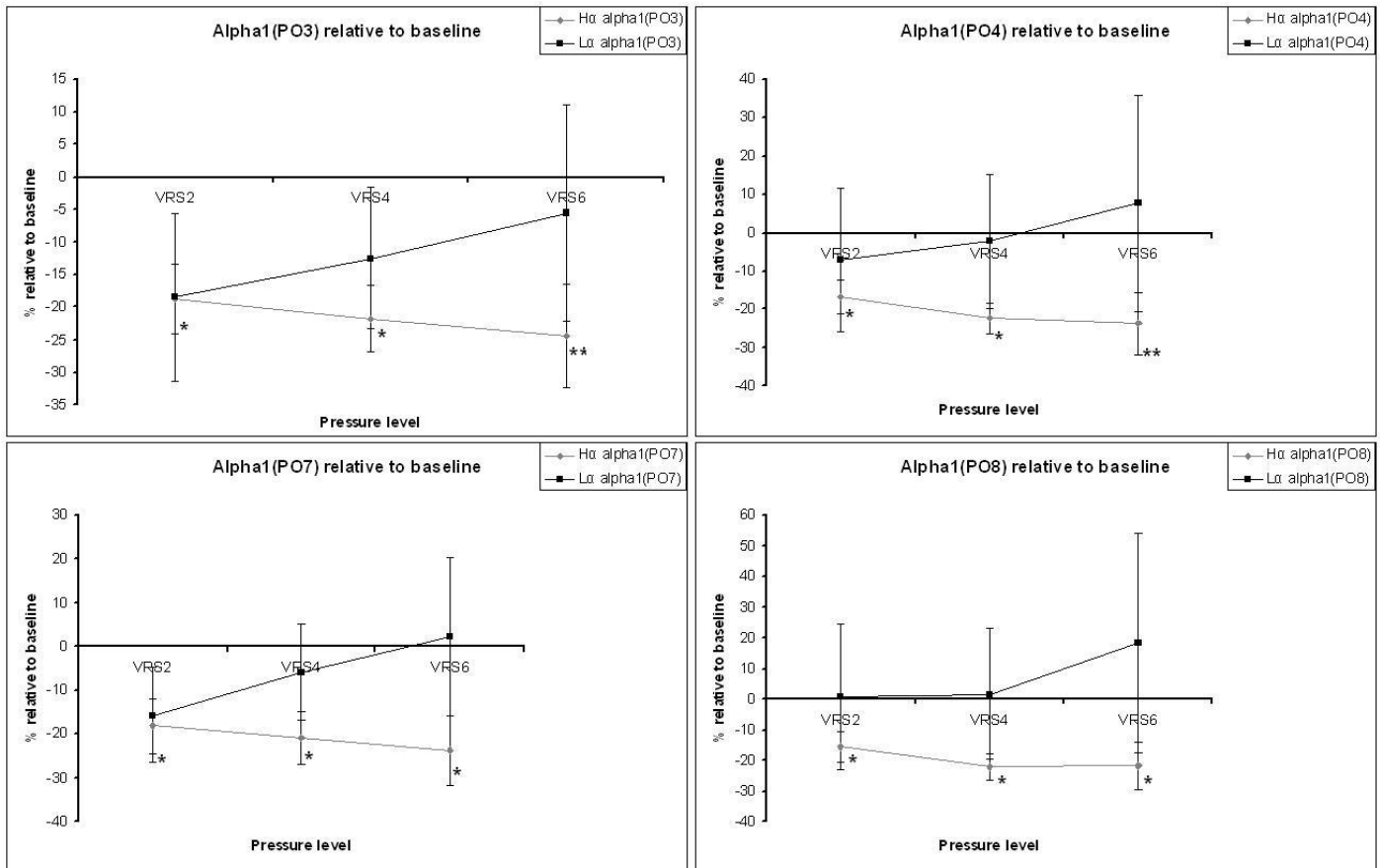


Figure 4: Significant differences between H_a (grey) and L_a (black) for α_1 (electrode location in brackets). Changes in VRS2, VRS4 and VRS6 are expressed relative to baseline. Statistical significance is marked with: * = $P < 0.05$, ** = $P < 0.01$

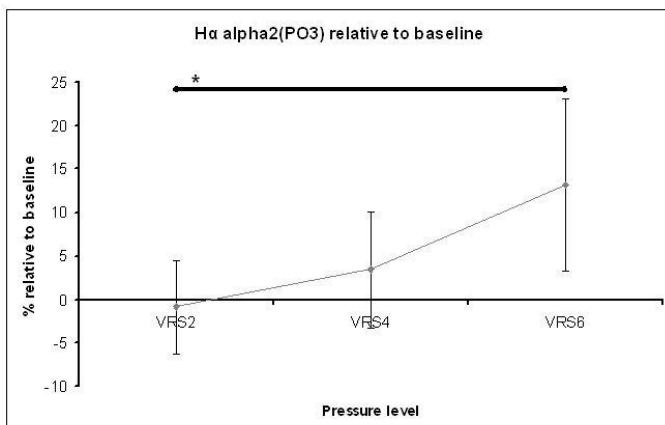


Figure 5: The significant $\alpha_2(PO3)$ EEG power increase from VRS2 to VRS4 ($P=0.009$) for the H_a . Changes in VRS2, VRS4 and VRS6 are expressed relative to baseline.

4.1.4. Pain-EEG relationships

The H_a did not show any significant relationship between alpha1 EEG activity and average subjective pain ratings as indicated in Figure 4 (alpha1(PO3):

$\text{pain} = 4.486 - 0.00308 * \alpha_1(\text{PO3})$, $R = 0.210$, $F = 2.677$, $P = 0.107$; alpha1(PO4):

$\text{pain} = 4.580 - 0.00364 * \alpha_1(\text{PO4})$, $R = 0.234$, $F = 3.350$, $P = 0.072$; alpha1(PO7):

$\text{pain} = 4.476 - 0.00323 * \alpha_1(\text{PO7})$, $R = 0.217$, $F = 2.285$, $P = 0.096$; alpha1(PO8):

$\text{pain} = 4.545 - 0.00349 * \alpha_1(\text{PO8})$, $R = 0.242$, $F = 3.601$, $P = 0.063$). The L_a

showed a significant positive relationship between alpha2(PO3) EEG activity and

average subjective pain ratings ($\text{pain} = 3.161 + 0.00919 * \alpha_2(\text{PO3})$, $R = 0.349$,

$F = 7.628$, $P = 0.008$) and no significant relationship between alpha2(PO4) and

average subjective pain ratings ($\text{pain} = 3.473 + 0.0054 * \alpha_2(\text{PO4})$, $R = 0.243$,

$F = 3.454$, $P = 0.068$) (Figure 6).

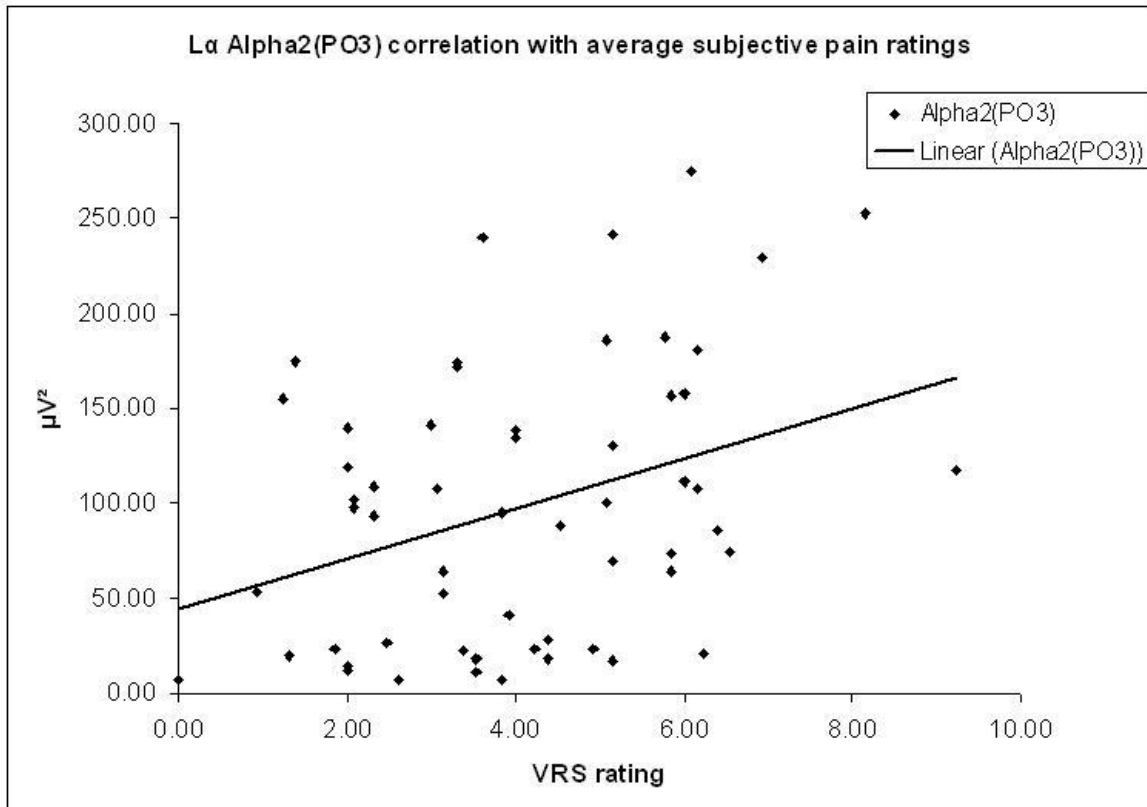


Figure 6:The significant ($P = 0.008$) positive correlation between alpha2(PO3) EEG activity and average subjective pain ratings for the low alpha group (L_a) is illustrated with the solid black line.

4.1.5. Degrees of unpleasantness and arousal

The H_{α} and L_{α} groups estimated their degrees of unpleasantness and negative arousal (Chang et al., 2002a) associated with tonic cuff-pressure pain after each experimental condition. The H_{α} group increased in the degree of unpleasantness between conditions VRS2 vs. VRS6 (-1.48 ± 0.26 vs. -2.96 ± 0.46 , $P < 0.001$) and VRS4 vs. VRS6 (-1.90 ± 0.31 vs. -2.96 ± 0.46 , $P = 0.003$). Further, the H_{α} group increased in the degree of negative arousal between conditions VRS2 vs. VRS6 (0.28 ± 0.44 vs. 2.07 ± 0.48 , $P < 0.05$). The L_{α} increased in the degree of unpleasantness between conditions VRS2 vs. VRS6 (-1 ± 0.43 vs. -2.51 ± 0.34 , $P < 0.05$) and in the degree of negative arousal between conditions VRS2 vs. VRS6 (0.85 ± 0.44 vs. 1.75 ± 0.48 , $P = 0.028$) (Figure 7).

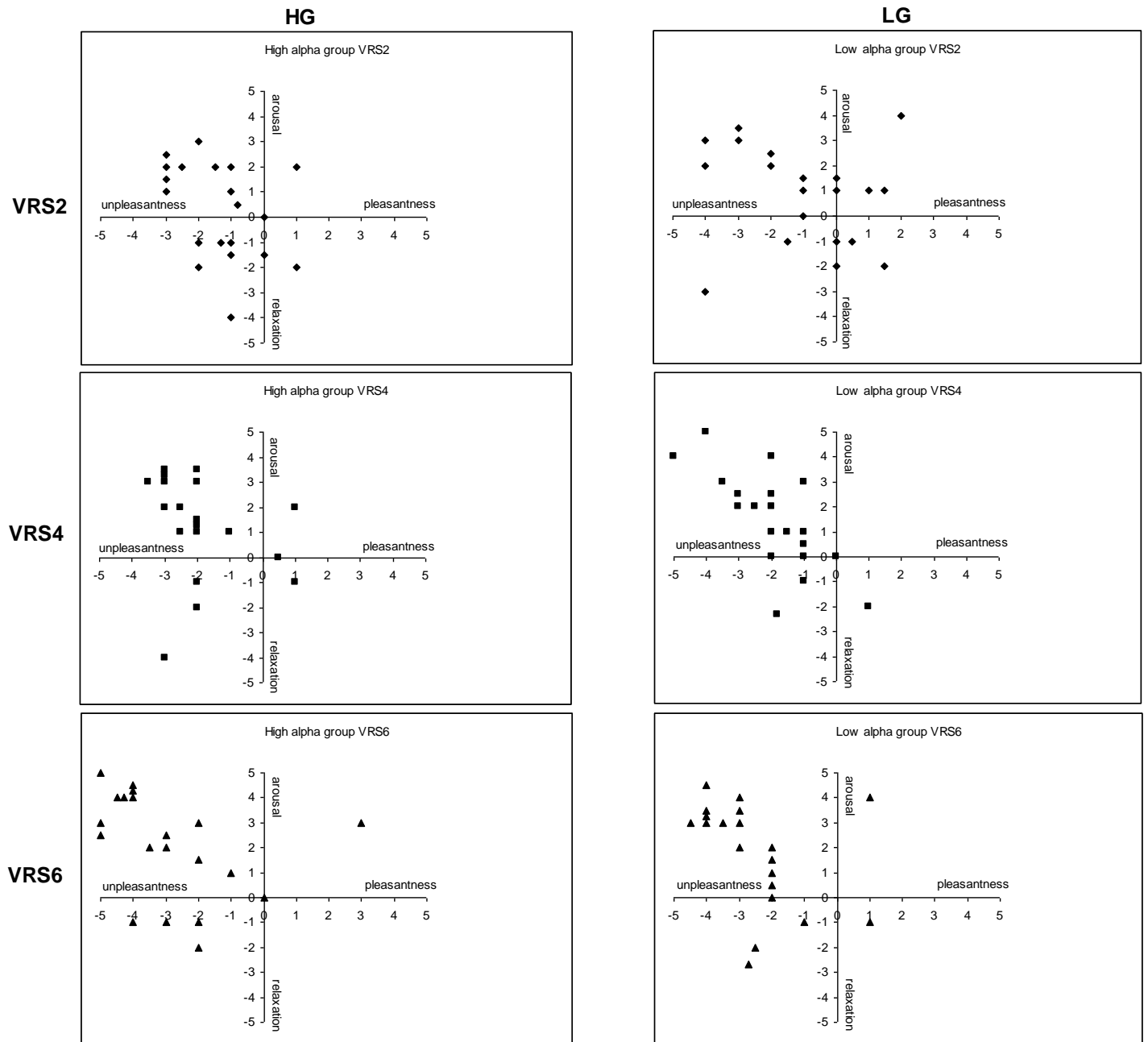


Figure 7: Degrees of negative arousal and unpleasantness associated with tonic cuff-pressure pain. In VRS2 the individual degrees of arousal and unpleasantness are marked with \diamond , in VRS4 the individual degrees of arousal and unpleasantness are marked with \blacksquare , and in VRS6 the individual degrees of arousal and unpleasantness are marked with \blacktriangle .

4.2. Gender differences in EEG responses to tonic pressure pain (study 2)

4.2.1. Gender effect in total EEG power

The differences between male and female subjects in EEG topography

(difference map, absolute power in μV^2) for all bands in all conditions are

illustrated in Figure 8. Gender differences were found in the delta band (total

activity across all experimental conditions) (F_{p2} : $F=15.189$, $P=0.034$, post

hoc <0.001 ; F_{p1} : $F=4.850$, $P=0.034$, post hoc $=0.034$) with the males exhibiting

higher activity than the females. The alpha2 band showed a significant difference

with the females having the highest power ($PO3$: $F=5.037$, $P=0.031$, post

hoc $=0.031$; $PO4$: $F=6.565$, $P=0.015$, post hoc $=0.015$). In the beta1 power the

females had higher activity than the males ($PO3$: $F=11.420$, $P=0.002$, post

hoc $=0.002$; $PO4$: $F=8.392$; $P=0.006$, post hoc $=0.006$).

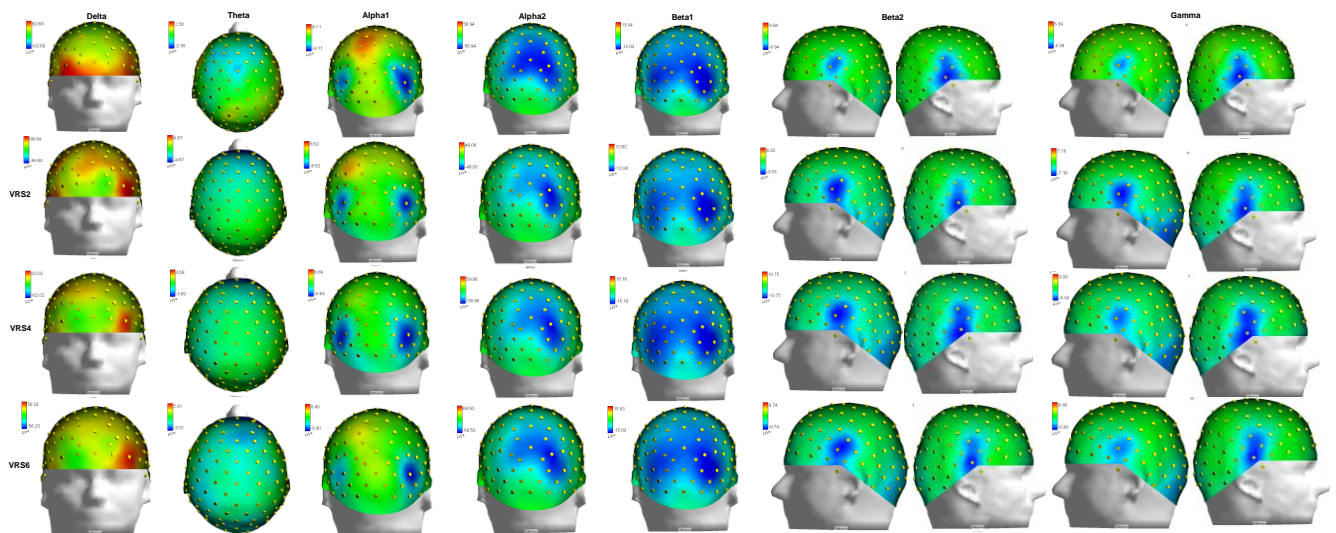


Figure 8: Difference map female subjects subtracted male subjects (absolute power maps for the female and male groups are presented in study 2). The differences between male and female subjects are illustrated in red (positive difference=males have higher power) and blue colors (negative difference=females have higher power). B = baseline, VRS2 = non-painful pressure level, VRS4 = slightly painful pressure level, pain threshold, and VRS6 = moderately painful pressure level.

4.2.2. Gender differences in EEG power during pain processing

Alpha2(PO3) shows gender differences during pain in baseline (B), VRS4, and VRS6 (gender x condition: $F=5.214$, $P=0.002$, post hoc: $B=0.007$, $VRS4=0.046$, $VRS6=0.041$), alpha2(PO4) showed gender differences in all pain conditions (gender x condition: $F=3.426$, $P=0.020$, post hoc: $B=0.005$, $VRS2=0.037$, $VRS4=0.018$, $VRS6=0.018$), both alpha2(PO3) and alpha2(PO4) powers the female group exhibiting higher powers than the male group. Beta2(T7) showed gender differences within the VRS4 condition with the female group having higher power in activity than the male group (gender x condition: $F=3.189$, $P=0.027$, post hoc= 0.018).

4.2.3. Gender differences in pain-EEG relationship

The males show a significant negative correlation between theta EEG activity and subjective pain ratings (Pearson's correlation coefficient= -0.261 , $P=0.0495$, Figure 9). The remaining EEG bands for the males did not show any relationship between EEG activity and subjective pain ratings. The female group did not show any relationship between EEG activity and subjective pain ratings.

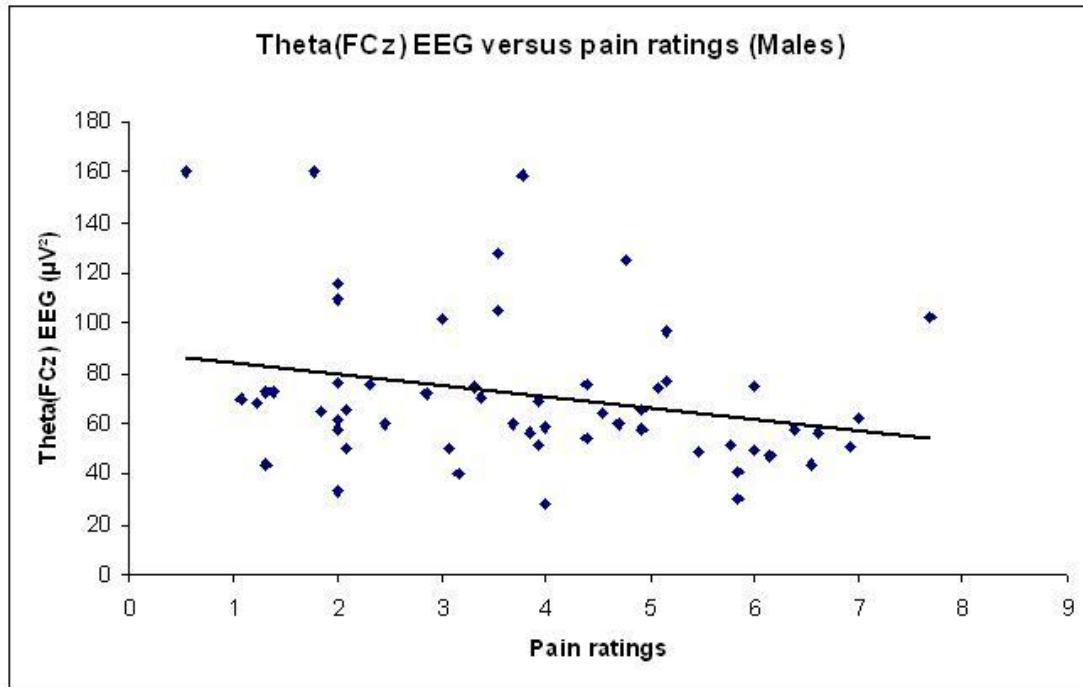


Figure 9: The theta(FCz) EEG for the males (pooled from all 3 experimental conditions) is negatively correlated with the verbal pain ratings pooled from all 3 experimental conditions (image taken from study 2).

4.2.3. Degrees of unpleasantness and arousal

The male and female groups estimated their degrees of unpleasantness and negative arousal (Chang et al., 2002a) associated with tonic cuff-pressure pain after each experimental condition and were significantly different in the degree of overall (difference between the two groups when all conditions were analyzed together) arousal (male vs. female: 0.86 ± 0.27 vs. 1.56 ± 0.27 , $P=0.043$). Further, the male and female groups had a significantly higher degree of unpleasantness between conditions VRS2 vs. VRS6 (males: -0.84 ± 0.33 vs. -2.75 ± 0.30 , $P<0.001$; females: -1.52 ± 0.37 vs. -2.66 ± 0.47 , $P \leq 0.05$, see Figure 10). Pooled data from

both groups showed significant differences in the degree of unpleasantness between experimental conditions VRS2 vs. VRS6 (-1.18 ± 0.25 vs. -2.71 ± 0.27 , $P < 0.05$) and VRS4 vs. VRS6 (-1.88 ± 0.21 vs. -2.71 ± 0.27 , $P < 0.05$) and in the degree of arousal between experimental conditions VRS2 vs. VRS6 (0.55 ± 0.30 vs. 1.88 ± 0.33 , $P < 0.05$).

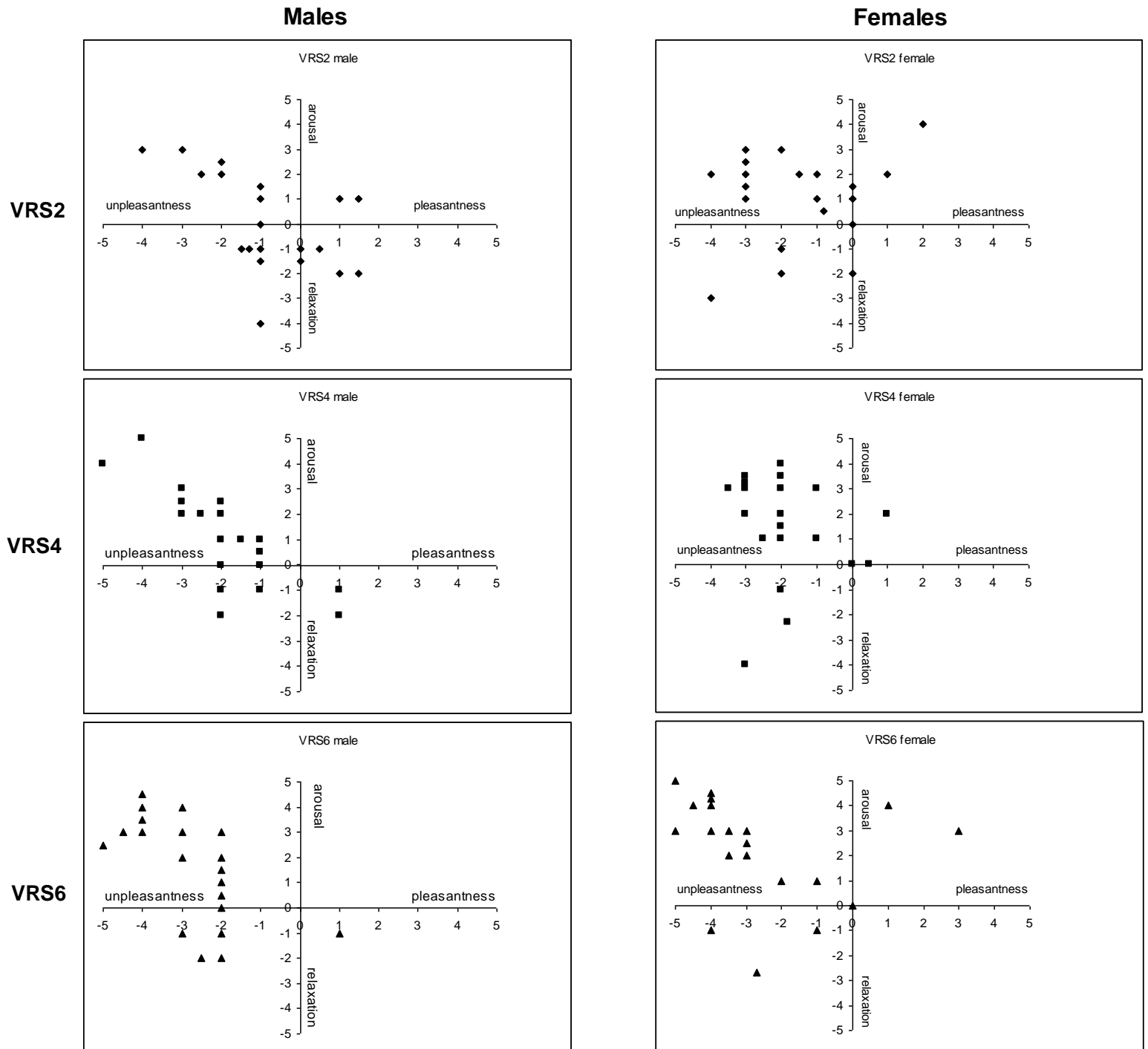


Figure 10: Degrees of negative arousal and unpleasantness associated with tonic cuff-pressure pain. In VRS2 the individual degrees of arousal and unpleasantness are marked with ◆, in VRS4 the individual degrees of arousal and unpleasantness are marked with ■, and in VRS6 the individual degrees of arousal and unpleasantness are marked with ▲.

4.3. Short-term cortical plasticity to shoulder muscle pain (study 3)

All subjects completed the experiment; however, 2 subjects were excluded from analysis because of large EEG artifacts, hence the analysis was based on 18 subjects.

4.3.1. Pre and post injection pain thresholds

The thenar injection group did not show any significant differences in pre and post glutamate injection trapezius electrical pain thresholds (PT_{single} : 6.8 ± 4.4 mA vs. 11.3 ± 7.6 mA, $t = -1.399$, $P = 0.195$; PT_{repeated} : 4.4 ± 2.8 mA vs. 10.0 ± 7.5 , $t = -1.203$, $P = 0.260$). No differences pre and post isotonic saline injection thresholds were found (PT_{single} : 5.1 ± 2.6 mA vs. 6.8 ± 3.2 , $t = -2.231$, $P = 0.053$; PT_{repeated} : 4.4 ± 2.2 mA vs. 6.1 ± 3.2 mA, $t = -1.705$, $P = 0.122$).

The trapezius injection group showed a significant difference between pre and post glutamate injection trapezius PT_{single} (11.0 ± 7.2 mA vs. 15.9 ± 7.9 mA, $t = -2.535$, $P = 0.032$) and PT_{repeated} (4.3 ± 2.4 mA vs. 8.1 ± 3.3 mA, $t = -3.539$, $P = 0.006$) (Fig 1). No differences in trapezius electrical pain thresholds pre and post isotonic saline injection were found (PT_{single} : 11.7 ± 7.6 mA vs. 14.7 ± 7.9 mA, $t = -1.926$, $P = 0.086$, PT_{repeated} : 4.8 ± 2.4 mA vs. 7.9 ± 4.2 mA, $t = -1.673$, $P = 0.129$).

4.3.2. Peak latency

The peak latency at N100 to single pulse stimulation showed an interaction between injection site and experimental condition ($F=3.048$, $P=0.015$), where the

SEPs during heterotopic tonic pain had a significantly longer N100 peak latency than SEPs during homotopic tonic pain (120.4 ± 7.8 vs. 96.2 ± 5.0 , post hoc HSD=0.034, see Figure 11). Further, the peak latency for repeat(5) at P200 showed a significant difference between homotopic tonic pain and heterotopic tonic pain where homotopic pain had a significantly longer peak latency than heterotopic pain (207.4 ± 7.3 vs. 181.4 ± 6.7 , post hoc HSD=0.020).

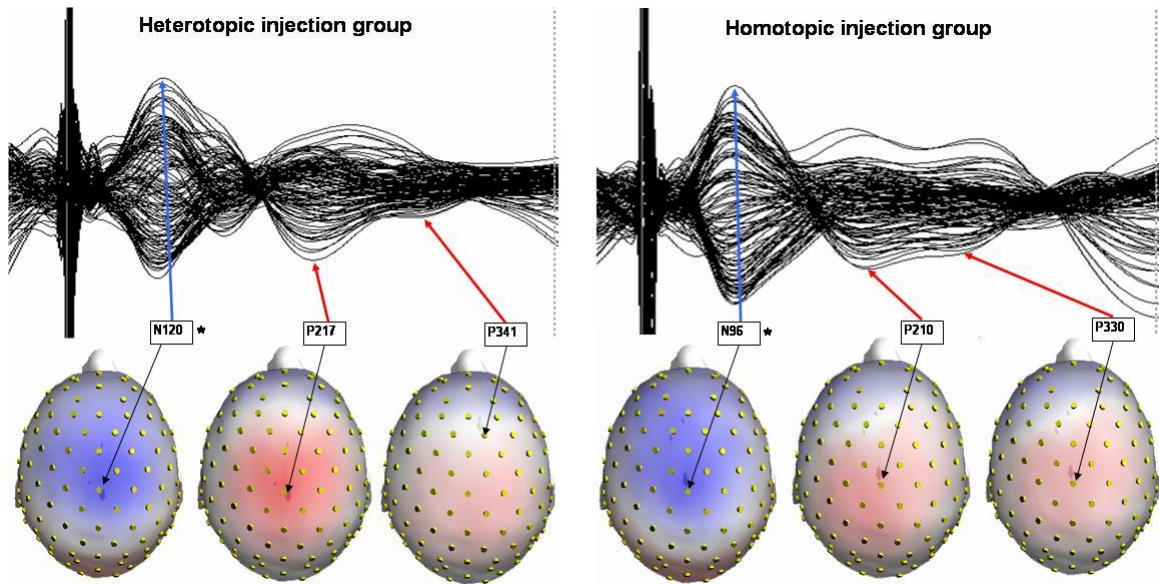


Figure 11: The compressed waveform for single pulse stimulation in the tonic pain condition (glutamate injection) for the heterotopic injection group (left) and the homotopic injection group (right) with the extracted peaks marked and the corresponding topography (image taken from study 3). The latency for the thenar injection group at N100 is longer than latency for the N100 component for the trapezius injection group. $P < 0.05$ is denoted with * (image taken from study 3).

4.3.3. Dipole localization

The y coordinate for repeat(1) stimulation showed for the P200 a significant interaction between injection site and experimental condition ($F=3.274$, $P=0.010$),

where the y coordinate was different during homotopic tonic pain compared to heterotopic tonic pain (homotopic: $y = 9.17$ mm vs. heterotopic: $y = -14.59$; post hoc HSD=0.024) and during homotopic sham pain and heterotopic sham pain (homotopic: $y = -12.56$ vs. heterotopic=12.10; post hoc HSD=0.024). The y coordinate for repeat(1) at P300 showed a significant shift between baseline and heterotopic tonic pain (baseline: $y = 9.34$ mm vs. heterotopic tonic pain: $y = -22.26$, post hoc HSD=0.041) (Figure 12). The z coordinate for repeat(1) at P300 showed a significant shift between homotopic tonic pain and post baseline (homotopic tonic pain: $z = -9.39$ mm vs. post baseline: $z = 11.74$ mm, post hoc HSD=0.037) (Figure 13).

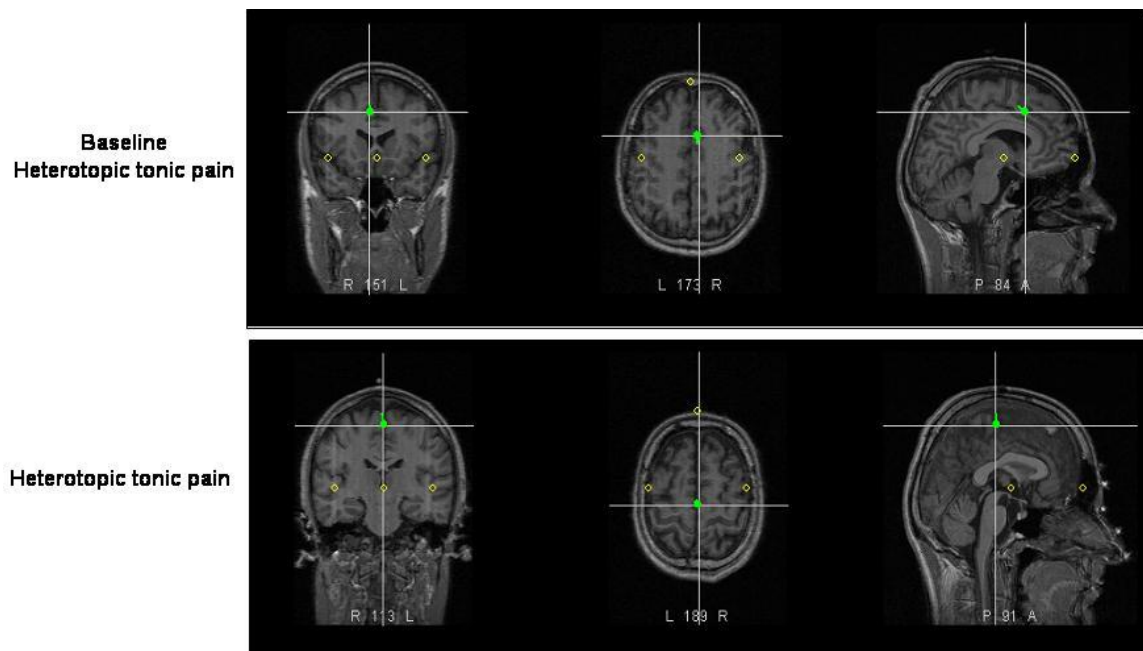


Figure 12: Changes in dipole localization (y-coordinate) from baseline to heterotopic tonic pain at P300 for repeat(1). At baseline the dipole was located in the cingulate gyrus and during heterotopic tonic pain the dipole was located in the superior frontal gyrus (image taken from study 3).

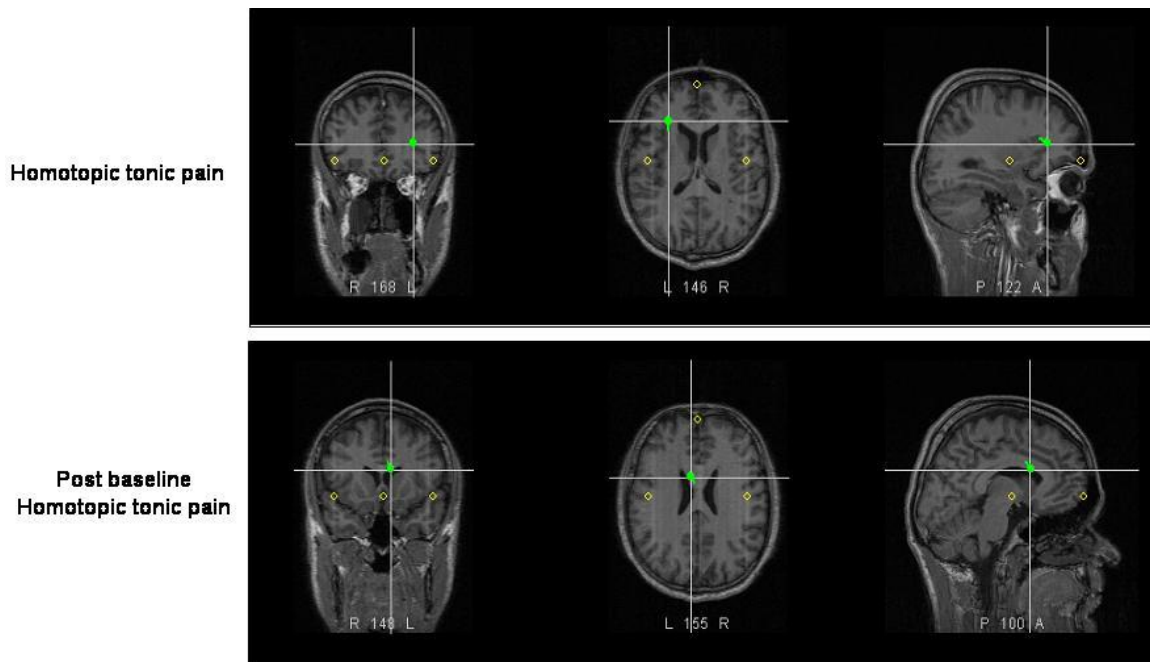


Figure 13: Changes in dipole localization (z-coordinate) from homotopic tonic pain to post baseline at P300 for repeat(1). During homotopic tonic pain the dipole was located in the superior temporal gyrus and at post baseline the dipole was located in the cingulate gyrus (image taken from study 3).

4.3.4. Dipole magnitude

There was a significant interaction ($F=2.347$, $P=0.049$) between muscle and experimental condition in current dipole magnitude for the train(5) stimulation at P300 but it was not confirmed by the post hoc test (post hoc >0.05).

4.4. Abnormal pain processing in tension type headache patients (study 4)

All participants completed the experiment, but three healthy controls were excluded because of large artefacts in the EEG data. The patients had been suffering from CTTH for a minimum of 1 year. Mean duration was 10.4 years (range 1- 25 years).

4.4.1. Electrical pain thresholds

There was no difference in PT_{single} (3.1 mA vs. 3.8 mA, $p = 0.4$) or in PT_{repeat} (1.2 mA vs. 2.1 mA, $p = 0.3$) between patients and controls.

4.4.2. Peak latency

There was no significant difference in peak latencies between patients and controls or between the baseline, tonic muscle pain and post- tonic muscle pain conditions.

4.4.3. Dipole localization

The dipole localization in patients at P200 for the 5th train stimulus was different ($F = 3.83$, $p = 0.03$, Post Hoc: y-coordinate, $p = 0.03$) from the localization in controls (patients: $y = 0.67$ mm; controls: $y = -19.79$ mm); but only at baseline recordings (Figure 14). During induced tonic muscle pain, no differences in the localizations of the dipoles between patients and controls were found ($p > 0.05$). Likewise, no difference in dipole localization (x, y, z) at N100, P200 or P300 between baseline and induced tonic muscle pain were found either in patients or in controls ($p > 0.05$).

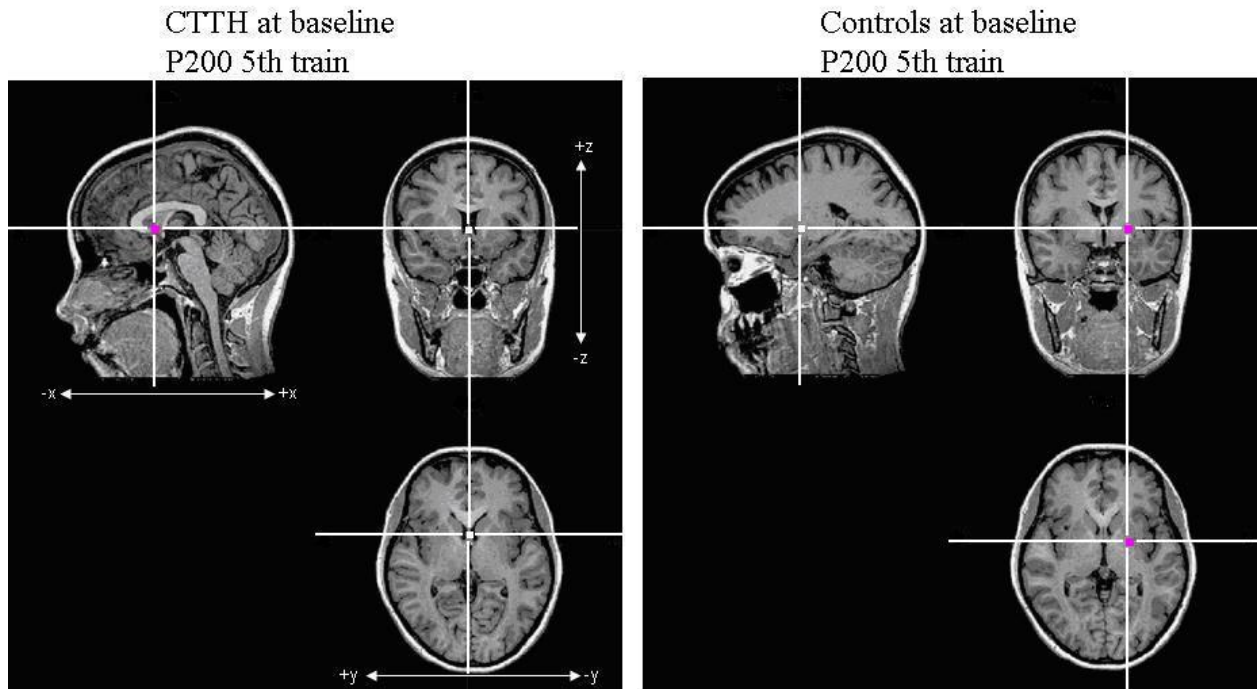


Figure 14: Baseline dipole localizations at P200 5th train. The marked lines intersect in the dipole.

Note: because the localization of the dipole for each group (CTTH and controls) is a calculated mean it is not constrained to a location within a gray-matter compartment (image taken from study 4).

4.4.4. Dipole magnitude

In controls, a reduction in magnitude between the conditions was found at the P200 dipole in response to both the 1st ($F = 3.3$, $p = 0.04$) and the 5th train stimuli ($F = 3.3$, $p = 0.04$) (Figure 15). Compared with baseline recordings the magnitude was lower during the tonic muscle pain condition (1st: $p = 0.001$; 5th: $p = 0.04$) and the post-tonic muscle pain condition (1st: $p = 0.002$; 5th: $p = 0.04$). This was in contrast to patients, where none of the post-hoc analyses showed significant differences in magnitude between the three conditions. At baseline, patients had a lower magnitude than controls at P200 according to the 1st train stimuli ($F = 3.3$, $p = 0.04$, Post Hoc: CTTH vs. controls = 0.01). In the tonic muscle pain and the

post-tonic muscle pain conditions there was no difference in magnitude of the dipoles between patients and controls.

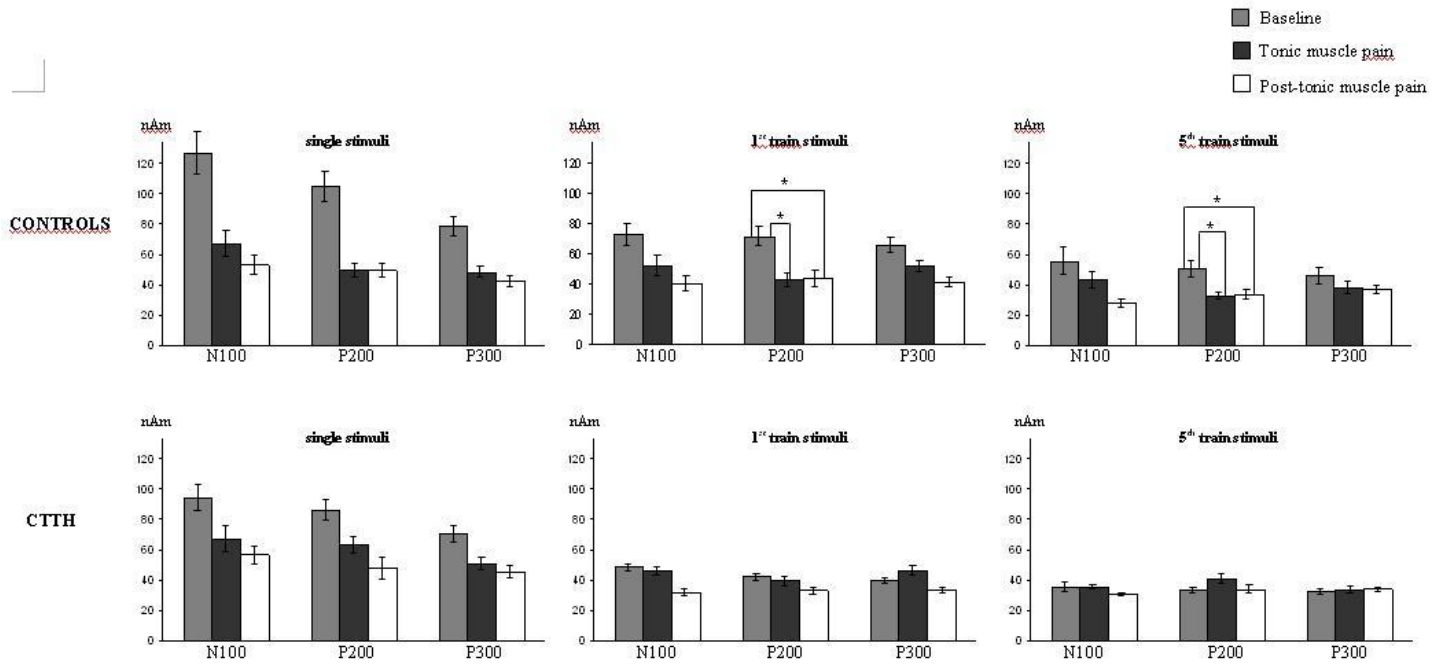


Figure 15: Magnitude of the dipoles (mean values \pm SE) in controls and patients at the three experimental conditions in response to single, 1st train and 5th train stimuli (image taken from study 4). * indicates significant difference at the 0.05 level.

5. Discussion

5.1. Pain-EEG relationships

Spontaneous EEG can reflect some aspects of pain processing since cerebral electrical activity can be changed when sensory information is processed in the brain. EEG frequency analysis may not allow comprehensive physiological interpretations, but each of the seven typical frequency bands (delta, theta, alpha1, alpha2, beta1, beta2, and gamma) can be related to functional aspects of pain processing.

5.1.1. Pain characteristics in low frequency EEG: delta and theta

EEG with a prefrontal focal maximum can be related to the novelty of attention and noxious stress on the eyes blinking and eyeball movement (Chang et al., 2001). Delta activity is usually also considered to be an expression of cortical inhibition (Ferracuti et al., 1994; Low, 2005). Ferracuti et al. (Ferracuti et al., 1994) suggested that their finding of increase in delta activity during cold pressor test may represent an attempt to inhibit sensorial perception of the nociceptive input. Increases in delta EEG power during cold pressor test have also been by Chang et al. (2002b) and Chen et al. (1989). Huber et al. (2006) also found increases in delta EEG power during tonic heat pain. We found that the males had higher delta EEG power than the females (study 2), however increasing delta EEG activity was not identified for the males or females. The higher delta EEG power for the males could imply activation of inhibitory processes which may reflect that men are less willing to report pain than women (Robinson et al., 2001).

Theta EEG activity responds selectively to the encoding of new information into episodic memory and reflects unspecific factors such as e.g. attentional demands, task difficulty and cognitive load (Klimesch, 1999). Theta activity in response to pain has been related to motivational regulation of the frontal cortex to produce habituation effects (Chang et al., 2002b). Dowman et al. (2008) found a decrease in the theta EEG amplitude during pain anticipatory cold pressor test when compared to arithmetic control condition which they suggested to be related to increases in working memory load. During tonic pain, decreases in theta EEG power have been documented in response to heat (Huber et al., 2006) and cold pressor test (Chang et al., 2002b; Chen et al., 1989); however there are inconsistent findings in the literature. In study 2 we found a negative relationship between theta EEG power and pain ratings for the males which may be related to encoding of new information in episodic memory (Klimesch, 1999) and habituation effects (Chang et al., 2002b).

5.1.2. Pain characteristics in middle frequency EEG: alpha1 and alpha2

Alpha is the dominant frequency in the human EEG. Alpha (alpha1 and alpha2) EEG rhythms are modulated by wakefulness or arousal (Cantero et al., 2002; Fumoto et al., 2004; Lindsey, 1960), speed of information processing (Surwillo, 1963a; Surwillo, 1963b), perception (Basar et al., 2000), motor functions (Pfurtscheller and Andrew, 1999), and pain (Chang et al., 2002a). Further, the

lower alpha band (alpha1) has been associated with attention processes where a decrease in alpha1 EEG activity reflects an increase in attention (Klimesch, 1999). The upper alpha band (alpha2) has been associated with retrieval processes in semantic memory (Klimesch, 1996). Alpha block has been reported to one of the main effects of tonic pain. Alpha block has been reported in response to cold pressor test (Chang et al., 2002b; Ferracuti et al., 1994; Chen et al., 1989; Dowman et al., 2008; Chen and Rappelsberger, 1994), injection of capsaicin (Chang et al., 2001), injection of hypertonic saline (Chang et al., 2003) and tonic heat pain (Huber et al., 2006). Huber et al. (2006) hypothesized that directing attention towards or away from pain affects the alpha EEG activity generated by the visual cortices. Subjects focusing on pain will exhibit an increase in posterior alpha and subjects attempting to cope with pain will exhibit a decrease in posterior alpha. This is in accord with our finding of a decrease in alpha1 EEG power for the female group (study 2) and the alpha1 desynchronization for the H_{α} (study 1) which may have a greater repertoire of pain-related coping strategies that include active behavioral and cognitive coping, avoidance, emotion-focused coping (see review Unruh, 1996). The increase of alpha2 EEG power for the male group (study 2) and the positive relationship between alpha2 EEG power and pain ratings for the L_{α} (study 1) may indicate increased information transfer and/or more attention towards painful stimulation. This attention may be selective attentional bias (vigilance) towards painful stimuli which mediates a negative reaction to pain and reduces their ability to cope with pain (Keogh et al., 2001b; Keogh et al., 2001a).

5.1.3. Pain characteristics in high frequency EEG: beta1, beta2 and gamma

Beta (beta1 and beta2) is a higher frequency activity and is characterized by cognitive and emotional processes (Ray and Cole, 1985), heightened vigilance in pain and discomfort (Chen et al., 1989) and scanning mechanisms that govern both perceptual and cognitive functions (Giannitrapani, 1971).

Increases in beta EEG activity have been related to alterations in sensory processing (Lalo et al., 2007). Pain broadly interferes with sensory, motor and cognitive processes and high beta activity may represent a physiological alerting function of pain (Ploner et al., 2004; Ploner et al., 2006). The increase of beta EEG power was also observed by Le Pera et al. (2000); the authors suggested that it was related to the emotional/attentional component of human pain responsiveness. Our results in study 2 are in accord with the results of Le Pera et al. and may indicate that the female groups' physiological alerting function is highly sensitive and activates coping responses to painful stimuli.

Gamma oscillations are particularly prominent during high vigilance. Gamma activity has band activity has been suggested to be task- and stimulus-related and to be involved in perceptual binding of multiple inputs (Engel and Singer, 2001). Gamma activity is also related to short-term working memory (Tallon-Baudry et al., 1998). It has been suggested that there is a relationship between alpha and gamma EEG activity. This relationship predicts a tonic experimental pain stimulus will produce a decrease in alpha and an increase in gamma EEG

amplitudes (Pfurtscheller, 1992; Edwards et al., 2005). Detectable changes in the gamma band were not found between the groups in studies 1 and 2.

5.2. Short-term cortical plastic changes measured by EEG source analysis

Cortical plasticity is a manifestation in many chronic pain syndromes (Flor, 2002a; Flor, 2002b; Knost et al., 1999) and has been studied by somatosensory evoked potentials (SEPs) (e.g. Shimojo et al., 2000; Wang et al., 2006; Waberski et al., 2007; Waberski et al., 2008; Hari and Forss, 1999; Murakami et al., 2008). Non-specific intra-muscular electrical stimulation (IMES) has been used in experimental studies to investigate cortical plasticity related to muscle pain (Niddam et al., 2005; Niddam et al., 2001; Niddam et al., 2007; Niddam et al., 2008; Svensson et al., 1997). Similar SEP topographies and waveforms are found for sensory inputs from skin and muscle and seem to be processed in nearly the same cerebral areas (Shimojo et al., 2000), although differences exist. Muscle SEPs does not contain detectable early SEP components (Niddam et al., 2005; Niddam et al., 2001), but has the first peak after 80-90 ms (Niddam et al., 2005). The middle components (50ms – 200ms) are diffuse distributed but they have been suggested to be compatible with A-delta myelinated fibers (Babiloni et al., 2001). The late components (200ms - 300ms) could partly be related to A-delta fiber activation (Chen, 2001). Further, SEPs from repeated painful muscle stimulation, as compared to single stimulation, decrease in amplitude at 100 ms (N100) and 250 ms (P250) and the P450 peak disappears (Chen et al., 2000).

5.2.1. Peak latency changes indicates changes in pain perception

It is generally agreed that the peak latency decreases as the stimulus intensity increases and that the peak latency increases as pain intensity decreases (Kakigi and Watanabe, 1996). Further, tonic muscle pain has shown to interfere with painful cutaneous somatosensory evoked potentials; both in latency and in amplitude (Valeriani et al., 2005). Findings of decreased latencies have been shown by Valeriani et al. (2008) who found that moderately painful IMES has a shorter latency at N120 than slightly painful and non-painful IMES. Decreased latencies have also been shown by Beitel and Dubner (1976) after application of noxious heat stimuli to a monkey's face and Shimoto et al. (2000) for reduction in P250 latency during painful intramuscular stimulation. In contrast, Babiloni et al. (2001) found longer latencies following painful galvanic stimulation as compared to non-painful galvanic stimulation. We found that heterotopic tonic shoulder muscle pain increased the latency of the N100 SEP (+24 ms, 20%) and that homotopic tonic shoulder muscle pain increased the latency of the P200 (+26 ms, +12.5%) (study 3). The prolonged peak latency for heterotopic tonic pain at N100 and for homotopic tonic pain at P200 suggest that both heterotopic and homotopic tonic induce pain relief. Further, this indicates that homotopic and heterotopic tonic painful counter stimulation modulate acute phasic pain differently. No changes in latency was found for the CTTH and the control group (study 4), however, these two groups did not get heterotopic but only homotopic tonic shoulder muscle pain and did not undergo a control session (sham pain); thus changes in latency could not be obtained.

5.2.2. Pain and cortical plasticity

Chronic pain patients often show lowered pain tolerance and thresholds related to the degree of chronicity. Cortical plastic changes may be involved in these alterations in sensitivity as well as peripheral and thalamic mechanisms (Flor, 2002a). It has been shown that chronic pain patients exhibit an expansion of the cortical representation zone related to nociceptive input and that this pain-related cortical plastic change develops over time (Flor, 2002a). However, short-term cortical plastic changes can also be detected in healthy volunteers after repeated phasic nociceptive inputs (e.g. Niddam et al., 2005; Babiloni et al., 2001; Niddam et al., 2001; Shimojo et al., 2000; Wang et al., 2006; Waberski et al., 2007; Waberski et al., 2008; Valeriani et al., 2005). We found that homotopic and heterotopic tonic pain modulated the y-coordinate of the P200 dipole differently (study 3). The P200 dipole component has been suggested to be an inhibitory process for irrelevant somatosensory information and involuntary motor responses (Babiloni et al., 2001). Changes in P300 dipole localization specific for homotopic and heterotopic tonic pain were also found. The P300 dipole is typically located around the cingulate gyrus (Bromm and Lorenz, 1998). The P300 dipole localization (z-coordinate) changed from homotopic tonic pain (superior temporal gyrus) to post baseline (cingulate gyrus) suggesting that homotopic tonic muscle pain counter stimulation can induce general long-lasting (during and after counter stimulation) short-term cortical plastic changes to painful intramuscular electrical stimulation which also was confirmed by the hypoalgesia present 20 minutes after tonic pain had disappeared. The P300 dipole localization (y-coordinate) changed from baseline (cingulate gyrus) to

heterotopic tonic pain (superior frontal gyrus) suggesting that ipsilateral heterotopic tonic muscle pain counter stimulation can induce local (only during counter stimulation) short-term cortical plastic changes to painful intramuscular electrical stimulation. Changes in dipole localization to nociceptive counter stimulation have not been reported so far. In fact, dipole localizations have been found to be identical with and without heterotopic counter stimulation (Naka et al., 1998; Dowman, 2002).

5.2.3. Dipole magnitude as measurement of deficient descending inhibition

Stimulus rate has been identified as a major factor influencing the source strengths i.e. dipole magnitude (e.g. Mauguiere et al., 1997); frequent and regular stimulus results in suppressed the middle to late SEP responses (Allison et al., 1992; Forss et al., 1995). Long (ISI > 3 sec) and/or random stimulus rate result in optimal late SEP responses because it allows a full recovery cycle for e.g. the SII and the posterior cingulate cortex (PPC) (Forss et al., 1994). With random stimulus rate and ISI \geq 4 sec; the stimulus rate should not have significant influence on the dipole magnitude. Further, no differences in dipole magnitude between homotopic vs. heterotopic tonic muscle pain were observed (study 3). This is in accordance with Niddam et al. (2001) who showed that dipole magnitudes most likely reflect the stimulus intensity rather than the modality of pain.

The observation of a reduction in magnitude of the dipoles from baseline to the tonic muscle pain and post-tonic muscle pain condition in controls but not in

patients (study 4) is the first report of abnormal supraspinal response to muscle pain in patients with CTTH. Moreover, it is the first evidence that the brain processing in patients with CTTH are different on a functional level from healthy controls. The reduction in magnitude of the dipoles between the conditions in controls but not in patients may be explained by deficient descending inhibition of the nociceptive input in patients. Deficient descending inhibition is also expected to play an important role in other chronic pain conditions and our finding is most likely not specific to CTTH.

5.4. EEG frequency analysis vs. source localization

Frequency analysis or source localization estimates alone does not tell the whole story about the brain functions involved in pain processing. Studies of spontaneous EEG typically utilize FFT power maps which are of considerable clinical interest for diagnosis of brain disease. Power maps are influenced by the overall levels of neural activity and may give a partly incorrect picture of brain function. Thus, estimates of the intracerebral sources may provide additional information about pain processing, however, this technique relies on the “inverse problem” which does not have a unique solution. The cerebrum generates rhythmic activity which often is not phase-locked to stimulus timing. This rhythmic activity is averaged out or eliminated when SEP data is averaged across trials (Laaksonen et al., 2008) although it may provide information about the neural activity not contained in the evoked responses (Salmelin et al., 2000). Hence, a combination of results from both methods may give us a more complete description.

EEG frequency analysis in response to tonic pain have reported relatively consistent changes; (a) increase in delta power; (b) rare changes in theta power; (c) decrease in alpha power; and (d) increase in beta power (for reviews see Chen, 2001; Bromm and Lorenz, 1998).

During tonic pain, the P200 dipole y-coordinate was different for the homotopic injection group as compared to the heterotopic injection group. The P200 dipole has been suggested to be involved in inhibition (Babiloni et al., 2001).

Heterotopic (Martikainen et al., 2004) and homotopic (Pud et al., 2005; 2006; Yarnitsky et al., 1997) counter stimulation have been indicated to have pain relieving effects. The mechanism of counter stimulation is generally explained by the gate control theory of pain inhibition (Melzack and Wall, 1965) and/or DNIC (Le Bars et al., 1979). Delta EEG activity originating from the frontal lobes is usually also considered to be an expression of cortical inhibition (Ferracuti et al., 1994; Low, 2005) and may represent an attempt to inhibit sensorial perception of the nociceptive input (Ferracuti et al., 1994). Hence, the change in the P200 dipole localization may also be related to alterations in delta EEG power.

Alpha EEG rhythms are assumed to arise in the thalamus and from here transmitted via thalamocortical tracts to the cortex (Schmidt, 1985). The alpha rhythms can be modified by inputs to the thalamus which synchronizes or desynchronizes the rhythmic alpha activity (Schmidt, 1985). It is generally agreed that the peak latency decreases as the stimulus intensity increases (Arendt-Nielsen, 1994). When stimulus intensity increases the subject becomes more

attentive towards the painful stimuli. Alerting a relaxed subject results in a desynchronization of the EEG where alpha activity decreases and beta activity increases. Further, alpha EEG activity has been associated with attention (Klimesch, 1999) and beta EEG activity has been related to alterations in sensory processing (Lalo et al., 2007). Changes in peak latency may also be related to alpha EEG desynchronization.

5.4.1. Source localization by FFT dipole approximation

Although sources are typically found close to the focal maxima or minima of the EEG power maps (Salmelin and Hamalainen, 1995), the relationship between cortical sources and EEG power spectra is difficult to identify. Power maps are influenced by the overall levels of neural activity and noise and may be slightly distorted in view of brain function and source analysis is based on assumptions and the solution of the inverse problem which does not have a unique solution. There are strengths and weaknesses in both approaches and they each provide different aspects of brain processing. Combining both methods and calculating the intracerebral dipole sources from the EEG FFT power maps (FFT dipole approximation method) is a technique which has been developing in the past two decades (e.g. Salmelin and Hamalainen, 1995; Lehmann and Michel, 1989; Lehmann and Michel, 1990; Michel et al., 1993, Zheng and Wan, 2005). The FFT approximation method is based on a map-oriented interpretation of the FFT coefficients and uses an optimization strategy (Lehmann and Michel, 1989). The Fast Fourier Transform of multichannel EEG data results in a sine and a cosine

coefficient for each electrode and each frequency point. These points are plotted in a sine-cosine diagram (NYQUIST) from where the phase information can be used to assign polarity to the amplitudes. The points plotted in the sine-cosine diagram typically form an ellipsoid-like pattern. A straight line is optimally approximated to these points in terms of phase angles. This is done by rotating the straight line around the mean of the sine-cosine points and calculating the orthogonal distances between the points and the line. These distances can be seen as inter-electrode voltages and be used to construct a potential distribution map. This potential map distribution is used for three-dimensional source localization by use of field theory (Kavanagh et al., 1978).

Dipole source analysis using the FFT approximation method has shown that the cortical generator of the human delta rhythm is located in the delta/theta band dipole is located anterior and deeper than the corresponding alpha band dipole (Michel et al., 1993). Further, the dipole localization for the delta/theta band was found to be significantly different from that of the alpha band, thus the authors concluded that different neural generator populations are involved in the generation of these different frequency components. However, the exact localizations of these dipoles were not specified. Further, localization of the alpha (alpha1 and alpha2) rhythm has been shown to be in the rolandic region and localization of the beta (beta1) rhythm has shown to be in the occipital region (Salmelin and Hamalainen, 1995). The FFT approximation method is an alternative to traditional time domain source analysis, however, it requires more processing time; it is also dependent on assumptions of the forward problem and

it is sensitive to band-pass filtering around the spectral maxima and FFT transformation lengths.

5.7. Methodological considerations

EEG has high temporal resolution and can provide information on a millisecond-by-millisecond basis and is suitable to study brain activity to brief phasic painful stimulation and to study the changes after painful conditions e.g. experimental tonic pain.

This thesis employed two different EEG analysis techniques: frequency analysis and source analysis.

5.1.1. Frequency analysis

EEG patterns are distinct for each individual which shows marked interindividual variations. Pain evokes physical as well as emotional aspects which eventually are reflected in the EEG. Spontaneous EEG has shown to change during e.g. fear, anxiety, attention and arousal and has a high degree of genetic determination (Vogel et al., 1979). This genetic variation in EEG indicates a corresponding variation in the function of the brain structure determining the EEG (Vogel et al., 1979). Frequency analysis does not consider temporal aspects but calculates an average power map over a chosen or measured time period. Power maps employ both spectral and spatial information but they do not allow comprehensive physiological interpretations, however, functional aspects of brain processing can be assessed.

5.1.2. Source analysis

The dipole sources are based on solving the inverse problem and it is based on many assumptions. These assumptions comprise a parametric model and this model makes it possible to obtain a unique solution, however, it is impossible to determine an arbitrary complex source distribution from a finite number of surface measurements (Kavanagh et al., 1978). Therefore, there are limitations and uncertainties of the inverse solution on which EEG source reconstruction is based. It is possible that the computed current sources corresponding to identical topographical maps may vary in location and strength (Shimojo et al., 2000). The position of the source is a rough indication of the center of gravity of the activated cortical area (Lopes da Silva et al., 1991). The inter-individual variability in the localization of the dipoles could most likely have been reduced by superimposing the dipoles on individual brain images and by using Polhemus (Polhemus FASTRACK®, www.polhemus.com) to mark the positions of the recording electrodes (and individual MRI) .

6. Concluding remarks

Treatment of pain is one of the major challenges in clinical medicine and the pain mechanisms in many diseases are poorly understood. Human experimental pain models allow the investigation of pain in controlled settings. The human brain and hence neuroimaging has become a major interest over the years. Human cerebral responses, both spontaneous EEG responses and somatosensory evoked responses, have been used to study pain processing. This thesis used spontaneous EEG responses and somatosensory evoked responses to study human pain processing according to two different pain models.

The results presented in this thesis indicate that EEG is a proper tool for investigating human pain and identifying differences in pain processing and/or stimulation modalities. EEG frequency power analysis in response to pain has proven to be useful to classify (high alpha and low alpha groups, study 1) and identify differences between groups (males and females, study 2). Further, it seems that there are two different phenomenon in pain-EEG relationships, gender and high/low alpha. The gender and high/low alpha phenomenon exhibit different EEG characteristics in response to tonic painful stimuli and EEG-pain rating correlations. These results may provide a new perspective of the differences that exist not only between male and female pain processing but also between groups which have different degrees of anxiety, vigilance, and fear towards pain.

EEG and somatosensory evoked potentials is able to show distinct differences between homotopic vs. heterotopic tonic pain and chronic pain patients vs. controls. Cortical plastic changes can be induced by experimental pain in healthy volunteers but not in patients which may be an indication of an existing cortical reorganization of the nociceptive system. These results may facilitate our understanding of human pain processing as well as inspire new approaches to assess/investigate chronic pain syndromes.

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